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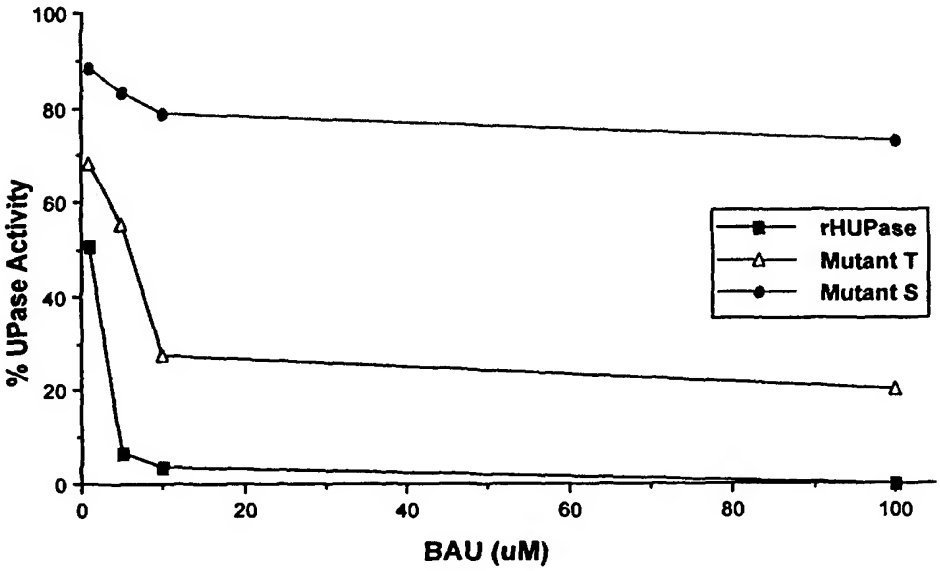

(57) Abstract: The present invention relates to human uridine phosphorylase nucleic acids and proteins encoded thereby. More specifically, the invention discloses that uridine phosphorylase in certain tumor tissues is resistant to inhibition by uridine phosphorylase inhibitor (UPI). The invention further relates to identification of mutations associated with uridine phosphorylase resistance to UPIs. These mutations include mutations located in exon 1 and exon 6. More specifically, the mutations include, but are not limited to, a change from a C to T at nucleotide 40, a C to T at nucleotide 135, a C to G at nucleotide 178, an A to C at nucleotide 182, a C to T at nucleotide 186, a G to A at nucleotide 713, and a G to A at nucleotide 752. The invention also relates to methods and kits relating to the nucleic and amino encoding UPase resistant to UPIs and uses therefor.

WO 01/60985 A2

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

TITLE
COMPOSITIONS, METHODS AND KITS RELATING TO URIDINE PHOSPHORYLASE GENE MUTATIONS

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority under 35 U.S.C. §119(e) to U.S. Provisional Application No. 60/182,273 filed on February 14, 2000.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

This invention was supported in part by funds from the U.S. Government (National Institutes of Health National Cancer Institute Grant No. RO1 CA67035) and the U.S. Government may therefore have certain rights in the invention.

BACKGROUND OF THE INVENTION

The invention relates to the discovery that uridine phosphorylase (UPase) of certain tumor tissues is resistant to inhibition by UPase inhibitors (UPIs), such as, *inter alia*, benzylacyclouridine (BAU), compared with the inhibition of wild type UPase by such UPIs. Further, the present invention relates to identification and characterization of mutations located in the nucleic acid encoding human UPase wherein the mutations are associated with UPI-resistance.

Moreover, identification of mutations in the nucleic acid encoding UPase resistant to BAU inhibition is important to the development of diagnostic and therapeutic procedures relating to detection and treatment of diseases, disorders or conditions in humans which are associated with UPI-resistant UPase.

Breast cancer represents the most common type of neoplasia in women. More than 150,000 new cases are diagnosed every year and more than 40,000 women will die of the disease. In the Western world, 1 in 8 women will develop breast cancer in their lifetime. The mortality rate has remained unchanged for the past 50-60 years and only

recently, with better detection technologies and improvements in treatment, has the mortality rate started to show significant improvement.

Approximately 5 to 10% of all breast cancers are linked to germ-line mutations. Mutations in BRCA1, a tumor suppressor gene located on chromosome 17, are believed to account for more of half of all the inherited breast cancers (Miki et al., 1994, Science 266:66-71; Futreal et al., 1994, Science 266:120-122). Analysis of families with hereditary breast cancer has helped to identify a second gene associated with breast cancer, termed BRCA2, which is located on chromosome 13 (Wooster et al., 1995, Nature 378:789-792).

Additional breast cancer related genes have been identified including germ-line mutations of the tumor suppressor gene p53 in families with Li-Fraumeni syndrome, the Cowden gene on chromosome 10, the gene for Ataxia-Telangiectasia and the proto-oncogene HRAS-1 as reviewed by Greene (1997, Mayo Clin. Proc. 72:54-65).

Somatic mutations of tumor suppressor genes with loss of their function has also been shown to play a role in breast carcinogenesis. Mutations in two main suppressor genes, the retinoblastoma gene (RB1) and the human P53 gene, have been identified in human breast cancer cells, and in other solid tumors (Clark et al., 1989, In: Growth Factors and Oncogenes, pages 167-172, Bolla et al., eds., Colloque INSERM/John Libbey Eurotext, France; Bartek et al. 1990, Oncogene 5:893–899; Lee et al., 1988, Science 241:218–221). Their mutations can lead to dysregulated transit of cells through the cell cycle and abnormal cell proliferation.

In addition to implicating mutations in certain wild type genes in breast cancer, studies have demonstrated that overexpression of certain oncogenes have been shown to induce or promote the malignant phenotype (Clark et al., 1989, In: Growth Factors and Oncogenes, vol. 190, pages 167-172, Bolla et al., eds., Colloque INSERM/John Libbey Eurotext, France). The products of oncogenes are frequently growth factors or growth-factor receptors. These oncogenes include members of the myc and ras family (*e.g.*, c-myc, Ha-ras-1), the family of receptors for epidermal growth factor (including erbB-1, HER2/neu or erbB-2), and the two more recent HER3 and HER4.

The growth factors that interact with these membrane receptors have not been identified completely.

Studies have demonstrated that UPase activity is elevated in tumor as compared to their normal tissue counterpart (*see, e.g.*, Liu et al., 1998, Cancer Res. 58:5418-5424). This phenomenon is also present when normal fibroblasts are transformed with c-H-ras (Grandori et al., 1997, Trends Biochem. 22:177-181).

Similarly, studies performed using an art-recognized rat liver carcinogenesis model (Lee et al., 1988, Biochim. Biophys. Acta, 942:139-149), demonstrated that UPase activity is up to 7-fold higher in the nodules than in the non-nodular surrounding liver or normal liver, as is the level of UPase protein as assessed using Western blot analysis, and the level of mRNA transcripts. Without wishing to be bound by any particular theory, these data suggest that disruption of uridine homeostasis in cells, such as variation in intracellular uridine transport and increase in UPase activity, have an effect or are linked to dramatic changes in cell growth regulation.

Further, expression of UPase has been shown to be induced in different tumor cell lines, such as Colon 26 and HCT-116, when in the presence of cytokines: TNF-α, IL-1α and IFN-α and -γ and vitamin D3 (Monks et al., 1983; Martin et al., 1989, Cancer Chemother. Pharmacol. 24: 9-14; Eda et al., 1993, Jpn. J. Cancer Res. 84: 341-347). Induction of UPase expression has also been reported in c-H-ras transformed NIH 3T3 cells resulting in an increased sensitivity to 5'-dFUrd (Watanabe et al., 1995, J. Biol. Chem. 270:12191-12196).

Some unusual promoter elements are present in the promoter region of the UPase gene as follows. Firstly, the promoter region of UPase comprises the consensus motifs for GATA-1 and 2 transcription factors. These factors mainly function as regulatory elements in the control of cellular differentiation of hematopoietic cells (Geng et al., 1991, Biochem. Pharm. 41:301-303). Secondly, another unusual promoter element present in the UPase gene promoter region is an IRF-1-like consensus element. This sequence is present just upstream (from about -21 to -33) of the putative transcription start site of the UPase gene and represents an important transcription factor in the

regulation of the interferon response system for infection, cell growth and apoptosis (Orkin et al., 1992, Blood 80: 575-581). Thirdly, two potential proto-oncogenes, C-Myb and V-Myb (Escalante et al., 1998, Nature. 391: 103-106), and a tumor suppressor gene p53 product binding site (Grandori et al., 1997, Trends Biochem. 22: 177-181) have been identified in the promoter region of UPase. Without wishing to be bound by any particular theory, since UPase is induced in various tumor tissues, the expression of UPase may be directly regulated by these proto-oncogenes and tumor suppressor gene products. Indeed, studies indicate that the binding of wild-type p53 to one of these motifs can inhibit the expression of the UPase gene following perturbation of the pyrimidine nucleotide pools.

UPase catalyzes the reversible phosphorolysis of uridine and to a lesser degree of thymidine. It also cleaves pyrimidine 2'- and 5'-deoxyribosides at a much lower rate (Langen et al., 1987, Biochem. Pharm. 16:1833-1837; Krenisky et al., 1964, J. Biol. Chem. 239:805-812; Krenisky et al., 1965, J. Biol. Chem. 240:1281-1286; Niedzwicki et al., 1981, Biochem. Pharmacol. 30: 2097-2101; Niedzwicki et al., 1982, Biochem. Pharmacol. 31:1857-1861). UPase affects activation and catabolism of fluoropyrimidines influencing their therapeutic capacity (Pontis et al., 1961, Biochim. Biophys. Acta 51:138-147; Birnie et al., 1963, Biochemistry 2:566-572; Woodman et al., 1980, Cancer Res. 40:507-511; Ishitsuka et al., 1980, Gann 71:112-123). Furthermore, UPase plays an important role in the homeostatic regulation of uridine concentration in plasma and tissues (Chu et al., 1984, Cancer Res. 44: 1852-1856; Darnowski et al., 1985, Cancer Res. 45:5364-5368; Chu et al., 1988, Nucleosides & Nucleotides 7:91-102; Monks et al., 1983, Biochem. Pharmacol. 32:2003-2009).

As mentioned previously elsewhere herein, UPase has a critical role in regulating the concentration of uridine in plasma and tissues (Chu et al., 1984, Cancer Res. 44:1852-1856; Darnowski et al., 1985, Cancer Res. 45:5364-5368; Chu et al., 1988, Nucleosides & Nucleotides 7: 91-102; Monks et al., 1983, Biochem. Pharmacol. 32:2003-2009). Uridine concentration is rigidly maintained at a concentration of 2-4 μM in plasma among different individuals and throughout different species (Muller-Tiemann, et

al., 1998, Proc. Natl. Acad. Sci. USA 95:6079-6084).

Besides catabolism by nucleoside phosphorylase activity, the intracellular concentration of uridine is regulated by its transport through the cell membranes. A facilitated-diffusion mechanism, which equilibrates intracellular and extracellular uridine, has been considered for many years to be responsible for intracellular uridine concentration. More recently, in addition to the nonconcentrative facilitated diffusion mechanism, several tissues were shown to possess a Na^+-dependent transporter for nucleosides (Darnowski, et al., 1985, Cancer Res. 45:5364-5368; Chu, et al., 1988, Nucleosides & Nucleotides 7: 91-102; Monks, et al., 1983, Biochem. Pharmacol. 32:2003-2009; Martin, et al., 1989, Cancer Chemother. Pharmacol. 24:9-14; Pizzorno, et al., 1998, Clin. Cancer Res. 4:1165-1175; Kuttesch, et al., 1982, Chemother. Pharmacol. 8:221-229; Kuttesch et al., 1982, Biochem. Pharmacol. 31:3387-3394).

Previous studies demonstrate the failure of all neoplastic cell lines *in vitro* and most tumors from clinical samples to concentrate uridine above media concentrations under normal growth conditions. These studies suggest that the ability to concentrate uridine may be an indication of the degree of differentiation in tumor cell lines. Alternatively, without wishing to be bound by any particular theory, the ability to concentrate uridine may play a role in the differentiation process. HL60 human leukemia cell line can be induced to differentiate to granulocytic or monocytic states by DMSO or phorbol ester (PMA), respectively. Under normal growth conditions, uridine entry is independent of Na^+ and a uridine gradient is not established; however, both of these differentiating agents induce the Na^+-dependent process, while, at the same time, facilitated diffusion and ^{3}H-NBMPR binding sites decrease. These changes occur within two days whereas full expression of the traditional markers of differentiation is not seen until three or four days (LeHir, et al., 1985, Pfugers Arch. 404:238-243).

Benzylacyclouridine (BAU, IND#039655) is a specific inhibitor of Uridine Phosphorylase (UPase) that has been utilized because of its ability to increase tissue uridine levels to reduce host toxicity and enhance the therapeutic index chemotherapeutic agents such as analogs of pyrimidine bases (*i.e.*, 5-fluorouracil (5-FU)

and pyrimidine nucleosides (*e.g.*, 3'-azido-3'-deoxythymidine [AZT]), by rescuing normal tissues (Darnowski et al., 1985, Cancer Res. 45:5364-5368; Chu et al., 1984, Cancer Res. 44: 1852-1856; Sommadossi et al., 1996, U.S. Patent No. 5, 567,689; Stolfi, 1996, U.S. Patent No. 5, 543,401; Naguib et al., 1992, U.S. Patent No. 5,141,943, Sommadossi et al., 1991, U.S. Patent No. 5,077,280), including preventing anemia induced by administration of AZT (Calabresi et al., 1990, U.S. Patent No. 4,950,466; Calabresi et al., 1989, U.S. Patent No. 4,874,602; Greer, 1988, European Patent Application No. EP0287128). It has been postulated that UPIs protect normal tissues against toxicity because of the elevation of plasma and intracellular levels of uridine.

There is a long felt need for the development of tumor diagnostics and therapeutics for breast, head-neck, and ovarian tumors. The present invention meets these needs and provides, among other things, methods for diagnosing and treating and for the development of therapeutic and diagnostic tools related to such tumors, which cause tremendous morbidity and mortality in humans.

BRIEF SUMMARY OF THE INVENTION

The invention includes isolated nucleic acids encoding human uridine phosphorylase wherein the uridine phosphorylase is resistant to a uridine phosphorylase inhibitor.

In one aspect, the uridine phosphorylase inhibitor is selected from the group consisting of benzylacyclouridine, benzyloxybenzylacyclouridine, aminomethyl-benzylacyclouridine, aminomethyl-benzyloxybenzylacyclouridine, hydroxymethyl-benzylacyclouridine, 5-(phenylselenyl)acyclouridine, 2',3',5'-tri-O-acetyluridine, 1-[(2-hydroxyethoxy)methyl]-5-phenylthiouracil, aryl-substituted 5-benzyluracils, 1-[(2-hydroxyethoxy)methyl]-5-benzyluracils, aryl-substituted 1-((2-hydroxyethoxy)methyl)-5-(3-phenoxybenzyl)uracil, 5-(benzyloxybenzyl)barbituric acid acyclonucleoside, and hydroxymethyl-benzyloxybenzylacyclouridine.

The invention further includes isolated nucleic acids encoding human uridine phosphorylase wherein the uridine phosphorylase is resistant to a uridine

phosphorylase inhibitor and further wherein the sequences of the nucleic acids comprise at least one mutation selected from the group consisting of a mutation located in exon 1 and a mutation located in exon 6.

The invention also includes isolated nucleic acids encoding human uridine phosphorylase, wherein the uridine phosphorylase is resistant to a uridine phosphorylase inhibitor and further wherein the sequences of the nucleic acids comprise at least one mutation selected from the group consisting of a C to T at nucleotide 40, a C to T at nucleotide 135, a C to G at nucleotide 178, an A to C at nucleotide 182, a C to T at nucleotide 186, a G to A at nucleotide 713, and a G to A at nucleotide 752.

In one aspect, the uridine phosphorylase inhibitor is selected from the group consisting of benzylacyclouridine, benzyloxybenzylacyclouridine, aminomethyl-benzylacyclouridine, aminomethyl-benzyloxybenzylacyclouridine, hydroxymethyl-benzylacyclouridine, 5-(phenylselenyl)acyclouridine, 2',3',5'-tri-O-acetyluridine, 1-[(2-hydroxyethoxy)methyl]-5-phenylthiouracil, aryl-substituted 5-benzyluracils, 1-[(2-hydroxyethoxy)methyl]-5-benzyluracils, aryl-substituted 1-((2-hydroxyethoxy)methyl)-5-(3-phenoxybenzyl)uracil, 5-(benzyloxybenzyl)barbituric acid acyclonucleoside, and hydroxymethyl-benzyloxybenzylacyclouridine.

The invention includes isolated human uridine phosphorylase polypeptides, wherein said polypeptides are resistant to a uridine phosphorylase inhibitor.

The invention further includes isolated human uridine phosphorylase polypeptides, wherein the polypeptides are resistant to a uridine phosphorylase inhibitor and further wherein the polypeptides are encoded by an isolated nucleic acid comprising at least one mutation selected from the group consisting of a mutation located in exon 1 and a mutation located in exon 6.

The invention includes isolated human uridine phosphorylase polypeptides, wherein the polypeptides are resistant to a uridine phosphorylase inhibitor and further wherein the polypeptides are encoded by an isolated nucleic acid comprising at least one mutation selected from the group consisting of a change from a C to T at nucleotide 40, a C to T at nucleotide 135, a C to G at nucleotide 178, an A to C at

nucleotide 182, a C to T at nucleotide 186, a G to A at nucleotide 713, and a G to A at nucleotide 752.

In one aspect, the uridine phosphorylase inhibitor is selected from the group consisting of benzylacyclouridine, benzyloxybenzylacyclouridine, aminomethyl-benzylacyclouridine, aminomethyl-benzyloxybenzylacyclouridine, hydroxymethyl-benzylacyclouridine, 5-(phenylselenyl)acyclouridine, 2',3',5'-tri-O-acetyluridine, 1-[(2-hydroxyethoxy)methyl]-5-phenylthiouracil, aryl-substituted 5-benzyluracils, 1-[(2-hydroxyethoxy)methyl]-5-benzyluracils, aryl-substituted 1-((2-hydroxyethoxy)methyl)-5-(3-phenoxybenzyl)uracil, 5-(benzyloxybenzyl)barbituric acid acyclonucleoside, and hydroxymethyl-benzyloxybenzylacyclouridine.

The invention includes isolated human uridine phosphorylase polypeptides, wherein the polypeptides are resistant to a uridine phosphorylase inhibitor and further wherein the amino acid sequences of the polypeptides comprise at least one mutation selected from the group consisting of a change from glutamic acid to lysine at amino acid residue number 121, and a change from valine to isoleucine at amino acid residue number 134.

The invention further includes antibodies that specifically bind with isolated human uridine phosphorylase polypeptides, wherein the polypeptides are resistant to a uridine phosphorylase inhibitor and further wherein the amino acid sequences of the polypeptides comprise at least one mutation selected from the group consisting of a change from glutamic acid to lysine at amino acid residue number 121, and a change from valine to isoleucine at amino acid residue number 134, or a fragment thereof.

The invention includes antibodies that specifically bind with isolated human uridine phosphorylase polypeptide or fragments thereof, wherein the polypeptides are resistant to a uridine phosphorylase inhibitor and further wherein the polypeptides are encoded by isolated nucleic acids comprising at least one mutation selected from the group consisting of a change from a C to T at nucleotide 40, a C to T at nucleotide 135, a

C to G at nucleotide 178, an A to C at nucleotide 182, a C to T at nucleotide 186, a G to A at nucleotide 713, and a G to A at nucleotide 752.

The invention includes isolated nucleic acids encoding human uridine phosphorylase wherein the uridine phosphorylase is resistant to a uridine phosphorylase inhibitor, and further wherein the nucleic acids comprise a nucleic acid encoding a tag polypeptide covalently linked thereto.

In one aspect, the tag polypeptide is selected from the group consisting of a myc tag polypeptide, a glutathione-S-transferase tag polypeptide, a green fluorescent protein tag polypeptide, a myc-pyruvate kinase tag polypeptide, a His6 tag polypeptide, an influenza virus hemagglutinin tag polypeptide, and a maltose binding protein tag polypeptide.

The invention includes isolated nucleic acids encoding human uridine phosphorylase, wherein the uridine phosphorylase is resistant to a uridine phosphorylase inhibitor and further wherein the sequences of the nucleic acids comprise at least one mutation selected from the group consisting of a C to T at nucleotide 40, a C to T at nucleotide 135, a C to G at nucleotide 178, an A to C at nucleotide 182, a C to T at nucleotide 186, a G to A at nucleotide 713, and a G to A at nucleotide 752, wherein the nucleic acid further comprises a nucleic acid encoding a tag polypeptide covalently linked thereto.

In one aspect, the tag polypeptide is selected from the group consisting of a myc tag polypeptide, a glutathione-S-transferase tag polypeptide, a green fluorescent protein tag polypeptide, a myc-pyruvate kinase tag polypeptide, a His6 tag polypeptide, an influenza virus hemagglutinin tag polypeptide, and a maltose binding protein tag polypeptide.

The invention includes isolated nucleic acids encoding a human uridine phosphorylase wherein the uridine phosphorylase is resistant to a uridine phosphorylase inhibitor, where the nucleic acids further comprise nucleic acids encoding a promoter/regulatory sequence operably linked thereto.

The invention further includes vectors comprising isolated nucleic acids encoding a human uridine phosphorylase wherein the uridine phosphorylase is resistant to a uridine phosphorylase inhibitor.

The invention also includes recombinant cells comprising isolated nucleic acids encoding a human uridine phosphorylase wherein the uridine phosphorylase is resistant to a uridine phosphorylase inhibitor.

The invention includes isolated nucleic acids encoding a human uridine phosphorylase, wherein the uridine phosphorylase is resistant to a uridine phosphorylase inhibitor and further wherein the sequences of the nucleic acids comprise at least one mutation selected from the group consisting of a C to T at nucleotide 40, a C to T at nucleotide 135, a C to G at nucleotide 178, an A to C at nucleotide 182, a C to T at nucleotide 186, a G to A at nucleotide 713, and a G to A at nucleotide 752, wherein the nucleic acids further comprise nucleic acids encoding a promoter/regulatory sequence operably linked thereto.

The invention includes vectors comprising isolated nucleic acids encoding a human uridine phosphorylase, wherein the uridine phosphorylase is resistant to a uridine phosphorylase inhibitor and further wherein the sequences of the nucleic acid comprise at least one mutation selected from the group consisting of a C to T at nucleotide 40, a C to T at nucleotide 135, a C to G at nucleotide 178, an A to C at nucleotide 182, a C to T at nucleotide 186, a G to A at nucleotide 713, and a G to A at nucleotide 752.

The invention also includes recombinant cells comprising isolated nucleic acids encoding a human uridine phosphorylase, wherein the uridine phosphorylase is resistant to a uridine phosphorylase inhibitor and further wherein the sequences of the nucleic acids comprise at least one mutation selected from the group consisting of a C to T at nucleotide 40, a C to T at nucleotide 135, a C to G at nucleotide 178, an A to C at nucleotide 182, a C to T at nucleotide 186, a G to A at nucleotide 713, and a G to A at nucleotide 752.

The invention includes transgenic non-human mammals comprising isolated nucleic acids encoding a human uridine phosphorylase wherein the uridine phosphorylase is resistant to a uridine phosphorylase inhibitor.

The invention further includes transgenic non-human mammals comprising isolated nucleic acids encoding a human uridine phosphorylase, wherein the uridine phosphorylase is resistant to a uridine phosphorylase inhibitor and further wherein the sequences of the nucleic acids comprise at least one mutation selected from the group consisting of a C to T at nucleotide 40, a C to T at nucleotide 135, a C to G at nucleotide 178, an A to C at nucleotide 182, a C to T at nucleotide 186, a G to A at nucleotide 713, and a G to A at nucleotide 752.

The invention includes isolated nucleic acids that hybridize with high stringency with nucleic acids encoding human uridine phosphorylase, wherein the isolated nucleic acids are selected from the group consisting of a nucleic acid having the sequence SEQ ID NO:3, a nucleic acid having the sequence SEQ ID NO:4, a nucleic acid having the sequence SEQ ID NO:5, a nucleic acid having the sequence SEQ ID NO:6, a nucleic acid having the sequence SEQ ID NO:7, and a nucleic acid having the sequence SEQ ID NO:8.

The invention includes methods for detecting the presence or absence of a nucleic acid encoding a human uridine phosphorylase resistant to a uridine phosphorylase inhibitor. The methods comprise:

(a) contacting a sample with a nucleic acid probe or primer which specifically hybridizes with the nucleic acid; and

(b) determining whether the nucleic acid probe or primer binds with a nucleic acid in the sample whereby when the nucleic acid probe or primer binds with a nucleic acid in the sample, the sample contains a nucleic acid which specifically hybridizes with the nucleic acid,

thereby detecting the presence or absence of the nucleic acid in a sample. In one aspect, the nucleic acid probe or primer is selected from the group consisting of a nucleic acid having the sequence SEQ ID NO:3, a nucleic acid having the sequence SEQ

ID NO:4, a nucleic acid having the sequence SEQ ID NO:5, a nucleic acid having the sequence SEQ ID NO:6, a nucleic acid having the sequence SEQ ID NO:7, and a nucleic acid having the sequence SEQ ID NO:8. In another aspect, the sample comprises mRNA molecules.

The invention also includes methods for detecting the presence or absence of a nucleic acid encoding a human uridine phosphorylase resistant to a uridine phosphorylase inhibitor in a biological sample. The methods comprise:

(a) amplifying a nucleic acid encoding a human uridine phosphorylase present in a biological sample wherein the nucleic acid comprises at least one mutation selected from the group consisting of a mutation located in exon 1 and a mutation located in exon 6;

(b) detecting any amplified target nucleic acid formed in (a) whereby when the nucleic acid is amplified the biological sample contains the nucleic acid which encodes a human uridine phosphorylase resistant to a uridine phosphorylase inhibitor, thereby detecting the presence or absence of a nucleic acid encoding a human uridine phosphorylase resistant to a uridine phosphorylase inhibitor in a biological sample. In one aspect, the mutation in exon 1 is selected from the group consisting of a change from a C to T at nucleotide 40, a C to T at nucleotide 135, a C to G at nucleotide 178, an A to C at nucleotide 182, and a C to T at nucleotide 186.

In another aspect, the mutation in exon 6 is selected from the group consisting of a change from G to A at nucleotide 713, and a change from G to A at nucleotide 752.

The invention includes kits for detecting a nucleic acid encoding human uridine phosphorylase resistant to a uridine phosphorylase inhibitor. The kits comprise at least one nucleic acid selected from the group consisting of a nucleic acid having the sequence SEQ ID NO:3, a nucleic acid having the sequence SEQ ID NO:4, a nucleic acid having the sequence SEQ ID NO:5, a nucleic acid having the sequence SEQ ID NO:6, a nucleic acid having the sequence SEQ ID NO:7, and a nucleic acid having the sequence SEQ ID NO:8, and an instructional material for the use thereof.

The invention further includes methods of detecting a cancer in a human. The method comprises obtaining a biological sample from the human, detecting the presence or absence of uridine phosphorylase resistant to a uridine phosphorylase inhibitor, wherein the presence of uridine phosphorylase resistant to a uridine phosphorylase inhibitor in the sample is an indication that the vertebrate animal has a cancer, thereby detecting a cancer in the human.

In one aspect, the cancer is selected from the group consisting of breast cancer, head-neck cancer, and ovarian cancer.

In another aspect, the biological sample is selected from the group consisting of a breast tissue sample, a head-neck tissue sample, and an ovarian tissue sample.

In a further aspect, detection is assessed using a method selected from the group consisting of a method of detecting uridine phosphorylase activity resistant to a uridine phosphorylase inhibitor and a method of detecting a mutation in a nucleic acid encoding uridine phosphorylase wherein the mutation is associated with uridine phosphorylase resistance to a uridine phosphorylase inhibitor.

In yet another aspect, the mutation is selected from a mutation in exon 1 and a mutation in exon 6. In a further aspect, the mutation in exon 1 is selected from the group consisting of a change from a C to T at nucleotide 40, a C to T at nucleotide 135, a C to G at nucleotide 178, an A to C at nucleotide 182, and a C to T at nucleotide 186.

In another aspect, the mutation in exon 6 is selected from the group consisting of a change from G to A at nucleotide 713, and a change from G to A at nucleotide 752.

In yet a further aspect, the mutation is detected using a nucleic acid selected from the group consisting of a nucleic acid having the sequence SEQ ID NO:3, a nucleic acid having the sequence SEQ ID NO:4, a nucleic acid having the sequence SEQ ID NO:5, a nucleic acid having the sequence SEQ ID NO:6, a nucleic acid having the sequence SEQ ID NO:7, and a nucleic acid having the sequence SEQ ID NO:8.

The invention includes methods of monitoring the treatment of a human

previously diagnosed with cancer. The methods comprise:

(a) assessing the level of uridine phosphorylase resistant to a uridine phosphorylase inhibitor in a first biological sample obtained from the human to determine an initial level of uridine phosphorylase resistant to a uridine phosphorylase inhibitor;

(b) administering an anti-cancer therapy to the human;

(c) assessing the level of uridine phosphorylase resistant to a uridine phosphorylase inhibitor in a second otherwise identical biological sample obtained from the human during or after the therapy;

(d) comparing the level of uridine phosphorylase resistant to a uridine phosphorylase inhibitor in the first biological sample with the level of uridine phosphorylase resistant to a uridine phosphorylase inhibitor in the second biological sample; and

(e) correlating any reduction in level of uridine phosphorylase resistant to a uridine phosphorylase inhibitor with the effectiveness of the anti-cancer therapy, thereby monitoring the treatment of a human previously diagnosed with cancer.

In one aspect, the methods further comprise repeating (b) through (e) during the course of the human's illness, anti-cancer therapy, or any period or portion thereof.

In another aspect, the level of uridine phosphorylase activity resistant to a uridine phosphorylase inhibitor is assessed using a method selected from the group consisting of a method of detecting uridine phosphorylase activity resistant to a uridine phosphorylase inhibitor and a method of detecting a mutation in a nucleic acid encoding uridine phosphorylase wherein the mutation is associated with uridine phosphorylase resistance to a uridine phosphorylase inhibitor.

The invention includes methods of identifying a human afflicted with a disease, disorder or condition associated with expression of uridine phosphorylase resistant to a uridine phosphorylase inhibitor. The methods comprise detecting a mutation in a nucleic acid encoding uridine phosphorylase in a human associated with

resistance to a uridine phosphorylase inhibitor, thereby detecting a human afflicted with a disease, disorder or condition associated with expression of uridine phosphorylase resistant to a uridine phosphorylase inhibitor.

The invention further includes methods of detecting a mutation in a uridine phosphorylase allele in a human. The methods comprise comparing the nucleic acid sequence encoding uridine phosphorylase resistant to a uridine phosphorylase inhibitor obtained from a tumor tissue sample obtained from a human with the nucleic acid sequence encoding uridine phosphorylase not resistant to a uridine phosphorylase inhibitor obtained from an otherwise identical non-tumor tissue sample obtained from the human, wherein any difference between the nucleic acid sequence encoding uridine phosphorylase resistant to a uridine phosphorylase inhibitor and the sequence encoding uridine phosphorylase not resistant to a uridine phosphorylase inhibitor detects a mutation in a uridine phosphorylase allele in the human.

The invention also includes methods of detecting a mutation in a uridine phosphorylase allele in a human. The methods comprise comparing the genomic nucleic acid sequence encoding uridine phosphorylase resistant to a uridine phosphorylase inhibitor with the genomic nucleic acid sequence encoding uridine phosphorylase not resistant to a uridine phosphorylase inhibitor, wherein any difference between the genomic nucleic acid sequence encoding uridine phosphorylase resistant to a uridine phosphorylase inhibitor and the genomic nucleic acid sequence encoding uridine phosphorylase not resistant to a uridine phosphorylase inhibitor detects a mutation in a uridine phosphorylase allele in the human.

The invention includes methods of treating cancer in a human receiving 5-fluorouracil. The methods comprise assessing the presence of uridine phosphorylase resistant to a uridine phosphorylase inhibitor in a tumor sample obtained from a human where the presence of uridine phosphorylase resistant to a uridine phosphorylase is an indication that the uridine phosphorylase inhibitor should be administered to the human, thereby treating a cancer in a human receiving 5-fluorouracil.

The invention includes kits for detecting a cancer in a human. The kits comprise at least one nucleic acid selected from the group consisting of a nucleic acid having the sequence SEQ ID NO:3, a nucleic acid having the sequence SEQ ID NO:4, a nucleic acid having the sequence SEQ ID NO:5, a nucleic acid having the sequence SEQ ID NO:6, a nucleic acid having the sequence SEQ ID NO:7, and a nucleic acid having the sequence SEQ ID NO:8, and an instructional material for the use thereof.

In one aspect, the cancer is selected from the group consisting of breast cancer, head-neck cancer, and ovarian cancer.

The invention also includes kits for monitoring the treatment of a human previously diagnosed with cancer. The kits comprise at least one nucleic acid selected from the group consisting of a nucleic acid having the sequence SEQ ID NO:3, a nucleic acid having the sequence SEQ ID NO:4, a nucleic acid having the sequence SEQ ID NO:5, a nucleic acid having the sequence SEQ ID NO:6, a nucleic acid having the sequence SEQ ID NO:7, and a nucleic acid having the sequence SEQ ID NO:8, and an instructional material for the use thereof.

The invention further includes kits for identifying a human afflicted with a disease, disorder or condition associated with expression of uridine phosphorylase resistant to a uridine phosphorylase inhibitor. The kits comprise at least one nucleic acid selected from the group consisting of a nucleic acid having the sequence SEQ ID NO:3, a nucleic acid having the sequence SEQ ID NO:4, a nucleic acid having the sequence SEQ ID NO:5, a nucleic acid having the sequence SEQ ID NO:6, a nucleic acid having the sequence SEQ ID NO:7, and a nucleic acid having the sequence SEQ ID NO:8, and an instructional material for the use thereof.

The invention includes kits for detecting a mutation in a uridine phosphorylase allele in a human. The kits comprise at least one nucleic acid selected from the group consisting of a nucleic acid having the sequence SEQ ID NO:3, a nucleic acid having the sequence SEQ ID NO:4, a nucleic acid having the sequence SEQ ID NO:5, a nucleic acid having the sequence SEQ ID NO:6, a nucleic acid having the

sequence SEQ ID NO:7, and a nucleic acid having the sequence SEQ ID NO:8, and an instructional material for the use thereof.

The invention also includes kits for treating cancer in a human receiving 5-fluorouracil. The kits comprise at least one nucleic acid selected from the group consisting of a nucleic acid having the sequence SEQ ID NO:3, a nucleic acid having the sequence SEQ ID NO:4, a nucleic acid having the sequence SEQ ID NO:5, a nucleic acid having the sequence SEQ ID NO:6, a nucleic acid having the sequence SEQ ID NO:7, and a nucleic acid having the sequence SEQ ID NO:8, and an instructional material for the use thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

The foregoing summary, as well as the following detailed description of the invention, will be better understood when read in conjunction with the appended drawings. For the purpose of illustrating the invention, there are shown in the drawings embodiment(s) which are presently preferred. It should be understood, however, that the invention is not limited to the precise arrangements and instrumentalities shown.

In the drawings:

Figure 1 is a graph depicting the increased level of UPase activity detected in tumor tissue compared with normal tissue for tumors obtained from various tissues, *i.e.*, breast, colon, kidney, liver, lung, ovarian, and small intestine.

Figure 2 is a graph depicting the level of inhibition of UPase activity by various doses (*i.e.*, 10 μM and 100 μM) of benzylacyclouridine (BAU) detected in UPase obtained from various tumors (*e.g.*, breast, head-neck, ovarian, kidney, lung, colon, thyroid, and adrenal tumor tissue).

Figure 3 is an image of gel depicting the loss of a SacI cleavage site in a nucleic acid encoding BAU-resistant UPase comprising a mutation (*i.e.*, transversion G to A at 713 bp).

Figure 4 is a graph depicting the resistance to BAU of normal wild type recombinant human UPase (rHUPase), UPase obtained from cells transfected with UPase

comprising a mutation at 713 bp, G to A (Mutant S), and UPase obtained from cells transfected with UPase comprising a mutation at 752 bp, G to A (Mutant T).

Figure 5 is an image of a gel depicting the mobility shift of UPase exon 6 comprising a mutation associated with BAU-resistant UPase. Briefly, polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) was performed using RNA obtained from BAU-resistant cells (*i.e.*, MDA-MB-468 and a human breast cancer 1T) and with RNA obtained from non-BAU-resistant UPase obtained from MCF-7 cells and normal tissue adjacent to the breast cancer (1N). The lower arrows indicate the shifted bands in MDA-MB-468 and human breast cancer 1T. Direct sequencing demonstrated that the mutation in MDA-MB-468 is a change from G to A at 713 bp while the mutation in breast cancer 1T is also a transversion from G to A but is located at 752 bp.

Figure 6 is an image depicting the nucleic acid sequence of normal wild type human *UPase* (SEQ ID NO:1) (GenBank Accession No. NM 003364). The mutations associated with UPI resistance are indicated by bold and underline and are as follows: a change from a C to T at nucleotide 40, a C to T at nucleotide 135, a C to G at nucleotide 178, an A to C at nucleotide 182, a C to T at nucleotide 186, a G to A at nucleotide 713, and a G to A at nucleotide 752.

Figure 7 is an image depicting the amino acid sequence of normal wild type human UPase (SEQ ID NO:2) (GenBank Accession No. CAA62369). The changes in amino acid associated with UPI resistance are indicated by bold and underline and are as follows: from a change from glutamic acid to lysine at amino acid residue number 121, and a change from valine to isoleucine at amino acid residue number 134.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides the nucleic acid and amino acid sequences of mutant human UPase, which sequences are important for development of diagnostics and therapeutics relating to various diseases, disorders and conditions. More specifically, it has been discovered, as disclosed herein, that UPase of various tumors, *e.g.*, breast, head-neck and ovarian, is resistant to the inhibitory effects of uridine phosphorylase

inhibitors, *e.g.*, benzylacyclouridine (BAU), compared to the UPase present in adjacent non-tumor tissues.

It has also been discovered, as disclosed herein, that certain mutations in the nucleic acid encoding UPase are associated with the UPI resistance. Therefore, the present invention provides novel diagnostics and therapeutics relating to detection and treatment of diseases, disorders and conditions associated with expression of UPI resistant UPase, including breast, head-neck and ovarian cancers.

Definitions

As used herein, each of the following terms has following meaning.

The articles "a" and "an" are used herein to refer to one or to more than one (*i.e.*, to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

As used herein, the term "adjacent" is used to refer to nucleotide sequences which are directly attached to one another, having no intervening nucleotides. By way of example, the pentanucleotide 5'-AAAAA-3' is adjacent the trinucleotide 5'-TTT-3' when the two are connected thus: 5'-AAAAATTT-3' or 5'-TTTAAAAA-3', but not when the two are connected thus: 5'-AAAAACTTT-3'.

As used herein, the phrase "allelic variant" refers to a nucleotide sequence which occurs at a given locus or to a polypeptide encoded by the nucleotide sequence.

As used herein, amino acids are represented by the full name thereof, by the three letter code corresponding thereto, or by the one-letter code corresponding thereto, as indicated in the following table:

Full Name	Three-Letter Code	One-Letter Code
Aspartic Acid	Asp	D
Glutamic Acid	Glu	E
Lysine	Lys	K
Arginine	Arg	R
Histidine	His	H

Tyrosine	Tyr	Y
Cysteine	Cys	C
Asparagine	Asn	N
Glutamine	Gln	Q
Serine	Ser	S
Threonine	Thr	T
Glycine	Gly	G
Alanine	Ala	A
Valine	Val	V
Leucine	Leu	L
Isoleucine	Ile	I
Methionine	Met	M
Proline	Pro	P
Phenylalanine	Phe	F
Tryptophan	Trp	W

As used herein, to "alleviate" a disease, disorder or condition mediated by or associated with uridine phosphorylase (UPase) resistant to a uridine phosphorylase inhibitor (UPI) means reducing the severity of the symptoms of the disease, disorder, or condition, such as, for example, various tumors. This includes, but is not limited to, methods whereby a UPI is administered with a chemotherapeutic agent such as, but not limited to, 5-fluorouracil (5-FU). This is because the UPI, by inhibiting UPase activity in normal tissues such that plasma and intracellular levels of uridine are maintained, protects normal tissues from the toxic effects of 5-FU, while the tumor cells, which comprise UPase which is not inhibited by UPI compared with normal tissues, is subject to the cytotoxic effects of 5-FU.

"Antisense" refers particularly to the nucleic acid sequence of the non-coding strand of a double stranded DNA molecule encoding a protein, or to a sequence which is substantially homologous to the non-coding strand. As defined herein, an

antisense sequence is complementary to the sequence of a double stranded DNA molecule encoding a protein. It is not necessary that the antisense sequence be complementary solely to the coding portion of the coding strand of the DNA molecule. The antisense sequence may be complementary to regulatory sequences specified on the coding strand of a DNA molecule encoding a protein, which regulatory sequences control expression of the coding sequences.

By the term "applicator" as the term is used herein, is meant any device including, but not limited to, a hypodermic syringe, a pipette, a nebulizer, and the like, for administering the a nucleic acid, protein, and/or composition of the invention to a mammal.

By "biological activity" or "activity" as the term is used herein, is meant that the UPase protein has the ability to effectuate its normal function(s) within the cell. More specifically, the biological activity of UPase encompasses, but is not limited to, phosphorolysis of uridine, uracil, and to a lesser extent, thymidine. Thus, the activity of UPase can be readily assessed using any of the methods disclosed herein, as well as any methods known in the art or to be developed in the future. That is, one skilled in the art would understand, based upon the disclosure provided herein, that there are a plethora of assays that can be used to asses UPase activity, both in the presence or absence of a UPI such as BAU, and that such assays include, but are not limited to, the assays described in, *e.g.*, Liu et al. (1998, Cancer Res. 58:5418-5424). The skilled artisan would further appreciate, based upon the instant disclosure, that the invention is not limited to any particular method of assessing the activity of UPase and that the invention encompasses any assay to assess the activity of UPase known in the art or to be developed in the future.

A "coding region" of a gene consists of the nucleotide residues of the coding strand of the gene and the nucleotides of the non-coding strand of the gene which are homologous with or complementary to, respectively, the coding region of an mRNA molecule which is produced by transcription of the gene.

A "coding region" of an mRNA molecule also consists of the nucleotide residues of the mRNA molecule which are matched with an anticodon region of a transfer

RNA molecule during translation of the mRNA molecule or which encode a stop codon. The coding region may thus include nucleotide residues corresponding to amino acid residues which are not present in the mature protein encoded by the mRNA molecule (*e.g.*, amino acid residues in a protein export signal sequence).

By "complementary to a portion or all of the nucleic acid encoding UPase" is meant a sequence of nucleic acid which does not encode UPase protein. Rather, the sequence which is identical to the non-coding strand of the nucleic acid encoding *UPase* and thus, does not encode UPase protein.

The terms "complementary" and "antisense" as used herein, are not entirely synonymous. "Antisense" refers particularly to the nucleic acid sequence of the non-coding strand of a double stranded DNA molecule encoding a protein, or to a sequence which is substantially homologous to the non-coding strand. "Complementary" as used herein refers to the broad concept of subunit sequence complementarity between two nucleic acids, *e.g.*, two DNA molecules. When a nucleotide position in both of the molecules is occupied by nucleotides normally capable of base pairing with each other, then the nucleic acids are considered to be complementary to each other at this position. Thus, two nucleic acids are complementary to each other when a substantial number (at least 50%) of corresponding positions in each of the molecules are occupied by nucleotides which normally base pair with each other (*e.g.*, A:T and G:C nucleotide pairs). As defined herein, an antisense sequence is complementary to the sequence of a double stranded DNA molecule encoding a protein. It is not necessary that the antisense sequence be complementary solely to the coding portion of the coding strand of the DNA molecule. The antisense sequence may be complementary to regulatory sequences specified on the coding strand of a DNA molecule encoding a protein, which regulatory sequences control expression of the coding sequences.

"Homologous" refers to the subunit sequence similarity between two polymeric molecules, *e.g.*, between two nucleic acid molecules, *e.g.*, two DNA molecules or two RNA molecules, or between two polypeptide molecules. When a subunit position in both of the two molecules is occupied by the same monomeric subunit, *e.g.*, if a

position in each of two DNA molecules is occupied by adenine, then they are homologous at that position. The homology between two sequences is a direct function of the number of matching or homologous positions, *e.g.*, if half (*e.g.*, five positions in a polymer ten subunits in length) of the positions in two compound sequences are homologous then the two sequences are 50% homologous, if 90% of the positions, *e.g.*, 9 of 10, are matched or homologous, the two sequences share 90% homology. By way of example, the DNA sequences 3'ATTGCC5' and 3'TATGCG5' share 50% homology.

A first oligonucleotide anneals with a second oligonucleotide with "high stringency" if the two oligonucleotides anneal under conditions whereby only oligonucleotides which are at least about 60%, more preferably at least about 65%, even more preferably at least about 70%, yet more preferably at least about 80%, and preferably at least about 90% or, more preferably, at least about 95%, complementary anneal with one another. The stringency of conditions used to anneal two oligonucleotides is a function of, among other factors, temperature, ionic strength of the annealing medium, the incubation period, the length of the oligonucleotides, the G-C content of the oligonucleotides, and the expected degree of non-homology between the two oligonucleotides, if known. Methods of adjusting the stringency of annealing conditions are known (*see, e.g.,* Sambrook et al., 1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York).

The determination of percent identity between two nucleotide or amino acid sequences can be accomplished using a mathematical algorithm. For example, a mathematical algorithm useful for comparing two sequences is the algorithm of Karlin and Altschul (1990, Proc. Natl. Acad. Sci. USA 87:2264-2268), modified as in Karlin and Altschul (1993, Proc. Natl. Acad. Sci. USA 90:5873-5877). This algorithm is incorporated into the NBLAST and XBLAST programs of Altschul, et al. (1990, J. Mol. Biol. 215:403-410), and can be accessed, for example, at the National Center for Biotechnology Information (NCBI) world wide web site having the universal resource locator "http://www.ncbi.nlm.nih.gov/BLAST/". BLAST nucleotide searches can be performed with the NBLAST program (designated "blastn" at the NCBI web site), using

the following parameters: gap penalty = 5; gap extension penalty = 2; mismatch penalty = 3; match reward = 1; expectation value 10.0; and word size = 11 to obtain nucleotide sequences homologous to a nucleic acid described herein. BLAST protein searches can be performed with the XBLAST program (designated "blastn" at the NCBI web site) or the NCBI "blastp" program, using the following parameters: expectation value 10.0, BLOSUM62 scoring matrix to obtain amino acid sequences homologous to a protein molecule described herein.

To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al. (1997, Nucleic Acids Res. 25:3389-3402). Alternatively, PSI-Blast or PHI-Blast can be used to perform an iterated search which detects distant relationships between molecules (*id.*) and relationships between molecules which share a common pattern. When utilizing BLAST, Gapped BLAST, PSI-Blast, and PHI-Blast programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used. *See* http://www.ncbi.nlm.nih.gov.

The percent identity between two sequences can be determined using techniques similar to those described above, with or without allowing gaps. In calculating percent identity, typically exact matches are counted.

As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules comprising an open reading frame encoding a polypeptide of the invention. Such natural allelic variations can typically result in 1-5% variance in the nucleotide sequence of a given gene. Alternative alleles can be identified by sequencing the gene of interest in a number of different individuals. This can be readily carried out by using hybridization probes to identify the same genetic locus in a variety of individuals. Any and all such nucleotide variations and resulting amino acid polymorphisms or variations that are the result of natural allelic variation and that do not alter the functional activity are intended to be within the scope of the invention.

A "coding region" of a gene consists of the nucleotide residues of the coding strand of the gene and the nucleotides of the non-coding strand of the gene which are homologous with or complementary to, respectively, the coding region of an mRNA

molecule which is produced by transcription of the gene.

A "coding region" of an mRNA molecule also consists of the nucleotide residues of the mRNA molecule which are matched with an anticodon region of a transfer RNA molecule during translation of the mRNA molecule or which encode a stop codon. The coding region may thus include nucleotide residues corresponding to amino acid residues which are not present in the mature protein encoded by the mRNA molecule (*e.g.*, amino acid residues in a protein export signal sequence).

"Encoding" refers to the inherent property of specific sequences of nucleotides in a polynucleotide, such as a gene, a cDNA, or an mRNA, to serve as templates for synthesis of other polymers and macromolecules in biological processes having either a defined sequence of nucleotides (*i.e.*, rRNA, tRNA and mRNA) or a defined sequence of amino acids and the biological properties resulting therefrom. Thus, a gene encodes a protein if transcription and translation of mRNA corresponding to that gene produces the protein in a cell or other biological system. Both the coding strand, the nucleotide sequence of which is identical to the mRNA sequence and is usually provided in sequence listings, and the non-coding strand, used as the template for transcription of a gene or cDNA, can be referred to as encoding the protein or other product of that gene or cDNA.

Unless otherwise specified, a "nucleotide sequence encoding an amino acid sequence" includes all nucleotide sequences that are degenerate versions of each other and that encode the same amino acid sequence. Nucleotide sequences that encode proteins and RNA may include introns.

"Expression vector" refers to a vector comprising a recombinant polynucleotide comprising expression control sequences operatively linked to a nucleotide sequence to be expressed. An expression vector comprises sufficient cis-acting elements for expression; other elements for expression can be supplied by the host cell or in an in vitro expression system. Expression vectors include all those known in the art, such as cosmids, plasmids (*e.g.*, naked or contained in liposomes) and viruses (*e.g.*, retroviruses, adenoviruses, and adeno-associated viruses) that incorporate the

recombinant polynucleotide.

As used herein, the term "fragment" as applied to a nucleic acid, may ordinarily be at least about 10 nucleotides in length, typically, at least about 20 nucleotides, more typically, from about 20 to about 50 nucleotides, preferably at least about 50 to about 100 nucleotides, even more preferably at least about 100 nucleotides to about 500 nucleotides, yet even more preferably at least about 500 to about 1,000, and most preferably, the nucleic acid fragment will be greater than about 1,340 nucleotides in length.

As used herein, the term "fragment" as applied to a polypeptide, may ordinarily be at least about seven contiguous amino acids, typically, at least about fifteen contiguous amino acids, more typically, at least about thirty contiguous amino acids, typically at least about forty contiguous amino acids, preferably at least about fifty amino acids, even more preferably at least about sixty amino acids and most preferably, the peptide fragment will be greater than about seventy contiguous amino acids in length.

As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences at least 60% (65%, 70%, preferably 75%) identical to each other typically remain hybridized with each other. Such stringent conditions are known to those skilled in the art and can be found in In: Current Protocols in Molecular Biology, at 6.3.1-6.3.6, John Wiley & Sons, N.Y. (1989). An example of stringent hybridization conditions are hybridization in 6× sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2× SSC, 0.1% SDS at 50-65°C. Preferably, an isolated nucleic acid molecule of the invention that hybridizes under stringent conditions to the sequence of any of SEQ ID NOs:1-8, and more preferably, to the sequence of any of SEQ ID NOs:3-8, or a complement thereof, corresponds to a naturally-occurring nucleic acid molecule. As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (*e.g.*, encodes a natural protein).

A "genomic DNA" is a DNA strand which has a nucleotide sequence

homologous with a gene. By way of example, both a fragment of a chromosome and a cDNA derived by reverse transcription of a mammalian mRNA are genomic DNAs.

"Homologous" as used herein, refers to the subunit sequence similarity between two polymeric molecules, *e.g.*, between two nucleic acid molecules, *e.g.*, two DNA molecules or two RNA molecules, or between two polypeptide molecules. When a subunit position in both of the two molecules is occupied by the same monomeric subunit, *e.g.*, if a position in each of two DNA molecules is occupied by adenine, then they are homologous at that position. The homology between two sequences is a direct function of the number of matching or homologous positions, *e.g.*, if half (*e.g.*, five positions in a polymer ten subunits in length) of the positions in two compound sequences are homologous then the two sequences are 50% homologous, if 90% of the positions, *e.g.*, 9 of 10, are matched or homologous, the two sequences share 90% homology. By way of example, the DNA sequences 3'ATTGCC5' and 3'TATGGC share 50% homology.

As used herein, "homology" is used synonymously with "identity."

In addition, when the term "homology" is used herein to refer to the nucleic acids and proteins, it should be construed to be applied to homology at both the nucleic acid and the amino acid levels.

As used herein, an "instructional material" includes a publication, a recording, a diagram, or any other medium of expression which can be used to communicate the usefulness of the composition of the invention for its designated use. The instructional material of the kit of the invention may, for example, be affixed to a container which contains the composition or be shipped together with a container which contains the composition. Alternatively, the instructional material may be shipped separately from the container with the intention that the instructional material and the composition be used cooperatively by the recipient.

An "isolated nucleic acid" refers to a nucleic acid segment or fragment which has been separated from sequences which flank it in a naturally occurring state, *e.g.*, a DNA fragment which has been removed from the sequences which are normally adjacent to the fragment, *e.g.*, the sequences adjacent to the fragment in a genome in

which it naturally occurs. The term also applies to nucleic acids which have been substantially purified from other components which naturally accompany the nucleic acid, *e.g.*, RNA or DNA or proteins, which naturally accompany it in the cell. The term therefore includes, for example, a recombinant DNA which is incorporated into a vector, into an autonomously replicating plasmid or virus, or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule (*e.g.*, as a cDNA or a genomic or cDNA fragment produced by PCR or restriction enzyme digestion) independent of other sequences. It also includes a recombinant DNA which is part of a hybrid gene encoding additional polypeptide sequence.

In the context of the present invention, the following abbreviations for the commonly occurring nucleic acid bases are used. "A" refers to adenosine, "C" refers to cytidine, "G" refers to guanosine, "T" refers to thymidine, and "U" refers to uridine.

"Derivatives," and "variants" of the peptides of the invention (or of the DNA encoding the same) are peptides which may be altered in one or more amino acids (or in one or more base pairs) such that the peptide (or DNA) is not identical to the sequences recited herein, but has the same property as the peptides disclosed herein, in that the peptide has the property of being more resistant to the inhibitory effect of a UPI than wild type UPase.

The term "mutation in a nucleic acid encoding UPase associated with resistance to a uridine phosphorylase inhibitor", as used herein, means a mutation in the coding region of a nucleic acid encoding UPase where the UPase is resistant to a UPI. Preferably, when the nucleic acid comprising the mutation is expressed in a cell, the UPase expressed therefrom is resistant to inhibition by a UPI, as detected using the methods disclosed herein, methods that are known in the art, or methods developed in the future for assessing inhibition of UPase activity by a compound.

By describing two polynucleotides as "operably linked" is meant that a single-stranded or double-stranded nucleic acid moiety comprises the two polynucleotides arranged within the nucleic acid moiety in such a manner that at least one of the two polynucleotides is able to exert a physiological effect by which it is characterized upon

the other. By way of example, a promoter operably linked to the coding region of a gene is able to promote transcription of the coding region.

Preferably, when the nucleic acid encoding the desired protein further comprises a promoter/regulatory sequence, the promoter/regulatory is positioned at the 5' end of the desired protein coding sequence such that it drives expression of the desired protein in a cell.

As used herein, the term "promoter/regulatory sequence" means a nucleic acid sequence which is required for expression of a gene product operably linked to the promoter/regulatory sequence. In some instances, this sequence may be the core promoter sequence and in other instances, this sequence may also include an enhancer sequence and other regulatory elements which are required for expression of the gene product. The promoter/regulatory sequence may, for example, be one which expresses the gene product in a tissue specific manner.

A "constitutive" promoter is a nucleotide sequence which, when operably linked with a polynucleotide which encodes or specifies a gene product, causes the gene product to be produced in a cell under most or all physiological conditions of the cell.

An "inducible" promoter is a nucleotide sequence which, when operably linked with a polynucleotide which encodes or specifies a gene product, causes the gene product to be produced in a cell substantially only when an inducer which corresponds to the promoter is present in the cell.

A "tissue-specific" promoter is a nucleotide sequence which, when operably linked with a polynucleotide which encodes or specifies a gene product, causes the gene product to be produced in a cell substantially only if the cell is a cell of the tissue type corresponding to the promoter.

The term "expression of a nucleic acid " as used herein means the synthesis of the protein product encoded by the nucleic acid.

The use of the term "DNA encoding" should be construed to include the DNA sequence which encodes the desired protein and any necessary 5' or 3' untranslated regions accompanying the actual coding sequence.

By the term "positioned at the 5' end" as used herein, is meant that the promoter/regulatory sequence is covalently bound to the 5' end of the nucleic acid whose expression it regulates, at a position sufficiently close to the 5' start site of transcription of the nucleic acid so as to drive expression thereof.

The direction of 5' to 3' addition of nucleotides to nascent RNA transcripts is referred to as the transcription direction. The DNA strand having the same sequence as an mRNA is referred to as the "coding strand"; sequences on the DNA strand which are located 5' to a reference point on the DNA are referred to as "upstream sequences"; sequences on the DNA strand which are 3' to a reference point on the DNA are referred to as "downstream sequences."

A "portion" of a polynucleotide means at least at least about twenty sequential nucleotide residues of the polynucleotide. It is understood that a portion of a polynucleotide may include every nucleotide residue of the polynucleotide.

A "polyadenylation sequence" is a polynucleotide sequence which directs the addition of a poly A tail onto a transcribed messenger RNA sequence.

A "polynucleotide" means a single strand or parallel and anti-parallel strands of a nucleic acid. Thus, a polynucleotide may be either a single-stranded or a double-stranded nucleic acid.

The term "nucleic acid" typically refers to large polynucleotides.

The term "oligonucleotide" typically refers to short polynucleotides, generally, no greater than about 50 nucleotides. It will be understood that when a nucleotide sequence is represented by a DNA sequence (i.e., A, T, G, C), this also includes an RNA sequence (*i.e.*, A, U, G, C) in which "U" replaces "T."

Conventional notation is used herein to describe polynucleotide sequences: the left-hand end of a single-stranded polynucleotide sequence is the 5'-end; the left-hand direction of a double-stranded polynucleotide sequence is referred to as the 5'-direction.

"Primer" refers to a polynucleotide that is capable of specifically hybridizing to a designated polynucleotide template and providing a point of initiation for synthesis of a complementary polynucleotide. Such synthesis occurs when the

polynucleotide primer is placed under conditions in which synthesis is induced, i.e., in the presence of nucleotides, a complementary polynucleotide template, and an agent for polymerization such as DNA polymerase. A primer is typically single-stranded, but may be double-stranded. Primers are typically deoxyribonucleic acids, but a wide variety of synthetic and naturally occurring primers are useful for many applications. A primer is complementary to the template to which it is designed to hybridize to serve as a site for the initiation of synthesis, but need not reflect the exact sequence of the template. In such a case, specific hybridization of the primer to the template depends on the stringency of the hybridization conditions. Primers can be labeled with, e.g., chromogenic, radioactive, or fluorescent moieties and used as detectable moieties.

"Probe" refers to a polynucleotide that is capable of specifically hybridizing to a designated sequence of another polynucleotide. A probe specifically hybridizes to a target complementary polynucleotide, but need not reflect the exact complementary sequence of the template. In such a case, specific hybridization of the probe to the target depends on the stringency of the hybridization conditions. Probes can be labeled with, *e.g.*, chromogenic, radioactive, or fluorescent moieties and used as detectable moieties.

"Recombinant polynucleotide" refers to a polynucleotide having sequences that are not naturally joined together. An amplified or assembled recombinant polynucleotide may be included in a suitable vector, and the vector can be used to transform a suitable host cell.

A recombinant polynucleotide may serve a non-coding function (*e.g.*, promoter, origin of replication, ribosome-binding site, etc.) as well.

A "recombinant polypeptide" is one which is produced upon expression of a recombinant polynucleotide.

"Polypeptide" refers to a polymer composed of amino acid residues, related naturally occurring structural variants, and synthetic non-naturally occurring analogs thereof linked via peptide bonds, related naturally occurring structural variants, and synthetic non-naturally occurring analogs thereof. Synthetic polypeptides can be

synthesized, for example, using an automated polypeptide synthesizer.

The term "protein" typically refers to large polypeptides.

The term "peptide" typically refers to short polypeptides.

Conventional notation is used herein to portray polypeptide sequences: the left-hand end of a polypeptide sequence is the amino-terminus; the right-hand end of a polypeptide sequence is the carboxyl-terminus.

As used herein, the term "reporter gene" means a gene, the expression of which can be detected using a known method. By way of example, the Escherichia coli *lacZ* gene may be used as a reporter gene in a medium because expression of the lacZ gene can be detected using known methods by adding the chromogenic substrate *o*-nitrophenyl-*β*-galactoside to the medium (Gerhardt et al., eds., 1994, Methods for General and Molecular Bacteriology, American Society for Microbiology, Washington, DC, p. 574). Similarly, as exemplified herein, luciferase can also be used as a reporter gene to detect expression of a protein of interest (*i.e.*, UPase) as can green fluorescent protein (GFP).

A "restriction site" is a portion of a double-stranded nucleic acid which is recognized by a restriction endonuclease.

A portion of a double-stranded nucleic acid is "recognized" by a restriction endonuclease if the endonuclease is capable of cleaving both strands of the nucleic acid at the portion when the nucleic acid and the endonuclease are contacted.

By the term "specifically binds," as used herein, is meant a compound, *e.g.*, a protein, a nucleic acid, an antibody, and the like, which recognizes and binds with a specific molecule, but does not substantially recognize or bind other molecules in a sample.

A first oligonucleotide anneals with a second oligonucleotide "with high stringency" if the two oligonucleotides anneal under conditions whereby only oligonucleotides which are at least about 60%, preferably at least about 65%, more preferably at least about 70%, even more preferably at least about 75%, and preferably at least about 90% or at least about 95%, complementary anneal with one another. The

stringency of conditions used to anneal two oligonucleotides is a function of, among other factors, temperature, ionic strength of the annealing medium, the incubation period, the length of the oligonucleotides, the G-C content of the oligonucleotides, and the expected degree of non-homology between the two oligonucleotides, if known. Methods of adjusting the stringency of annealing conditions are known (*see, e.g.*, Sambrook et al., 1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York).

As used herein, the term "substantially pure" describes a compound, *e.g.*, a nucleic acid, protein or polypeptide, which has been separated from components which naturally accompany it. Typically, a compound is substantially pure when at least about 10%, preferably at least about 20%, more preferably at least about 50%, still more preferably at least about 75%, even more preferably at least about 90%, and most preferably at least about 99% of the total material (by volume, by wet or dry weight, or by mole percent or mole fraction) in a sample is the compound of interest. Purity can be measured by any appropriate method, *e.g.*, by column chromatography, gel electrophoresis or HPLC analysis.

A compound, *e.g.*, a nucleic acid, a protein or polypeptide is also "substantially purified" when it is essentially free of naturally associated components or when it is separated from the native contaminants which accompany it in its natural state. Thus, a "substantially pure" preparation of a nucleic acid, as used herein, refers to a nucleic acid sequence which has been purified from the sequences which flank it in a naturally occurring state, *e.g.*, a DNA fragment which has been removed from the sequences which are normally adjacent to the fragment in a genome in which it naturally occurs.

Similarly, a "substantially pure" preparation of a protein or a polypeptide, as used herein, refers to a protein or polypeptide which has been purified from components with which it is normally associated in its naturally occurring state. A substantially pure peptide can be purified by following known procedures for protein purification, wherein an immunological, enzymatic or other assay is used to monitor

purification at each stage in the procedure. Protein purification methods are well known in the art, and are described, for example in Deutscher et al. (1990, In: Guide to Protein Purification, Harcourt Brace Jovanovich, San Diego).

By the term "exogenous nucleic acid" is meant that the nucleic acid has been introduced into a cell or an animal using technology which has been developed for the purpose of facilitating the introduction of a nucleic acid into a cell or an animal.

A cell that comprises an exogenous nucleic acid is referred to as a "recombinant cell." Such a cell may be a eukaryotic cell or a prokaryotic cell. A gene which is expressed in a recombinant cell wherein the gene comprises a recombinant polynucleotide, produces a "recombinant polypeptide."

By "tag" polypeptide is meant any protein which, when linked by a peptide bond to a protein of interest, may be used to localize the protein, to purify it from a cell extract, to immobilize it for use in binding assays, or to otherwise study its biological properties and/or function. A chimeric (*i.e.*, fusion) protein containing a "tag" epitope can be immobilized on a resin which binds the tag. Such tag epitopes and resins which specifically bind them are well known in the art and include, for example, tag epitopes comprising a plurality of sequential histidine residues (His6), which allows isolation of a chimeric protein comprising such an epitope on nickel-nitrilotriacetic acid-agarose, a hemagglutinin (HA) tag epitope allowing a chimeric protein comprising such an epitope to bind with an anti-HA-monoclonal antibody affinity matrix, a myc tag epitope allowing a chimeric protein comprising such an epitope to bind with an anti-myc-monoclonal antibody affinity matrix, a glutathione-S-transferase tag epitope, and a maltose binding protein (MBP) tag epitope, which can induce binding between a protein comprising such an epitope and a glutathione- or maltose-Sepharose column, respectively. Production of proteins comprising such tag epitopes is well known in the art and is described in standard treatises such as Sambrook et al., 1989, and Ausubel et al., *supra*. Likewise, antibodies to the tag epitope (*e.g.*, anti-HA, anti-myc antibody 9E10, and the like) allow detection and localization of the fusion protein in, for example, Western blots, ELISA assays, and immunostaining of cells.

As used herein, to "treat" means reducing the frequency with which symptoms are experienced by a patient.

"Uridine phosphorylase inhibitor" or "UPI", means any compound that inhibits the activity of uridine phosphorylase. There are a plethora of UPIs known in the art such as those described in, among others, Ashour et al. (2000, Cancer Chemother. Pharmacol. 46:235-240), el Khouni et al. (2000, Biochem. Pharmacol. 60:851-856), Ashour et al. (2000, Biochem. Pharmacol. 60:687-692), Ashour et al. (2000, Biochem. Pharmacol. 60:427-431), Ashour et al. (2000, Cancer Chemother. Pharmacol. 45:351-361), Orr et al., (1997, J. Med. Chem. 40:1179-1185), Sommadossi and el Kouni (1996, U.S. Patent. No. 5,567,689), Orr et al. (1995, J. Med. Chem. 38:3850-3856), Darnowski et al. (1985, Cancer Res. 45:5364-5368), Chu et al. (1984, Cancer Res. 44: 1852-1856), Stolfi (1996, U.S. Patent No. 5, 543,401), Naguib et al. (1992, U.S. Patent No. 5,141,943), Sommadossi et al. (1991, U.S. Patent No. 5,077,280), and as disclosed herein.

More specifically, suitable uridine phosphorylase inhibiting compounds include, but are not limited to, benzylacyclouridine (BAU); benzyloxybenzylacyclouridine (BBAU); aminomethyl-benzylacyclouridine (AMBAU); aminomethyl-benzyloxybenzylacyclouridine (AMB-BAU); hydroxymethyl-benzylacyclouridine (HMBAU); 5-(phenylselenyl)acyclouridine (PSAU); 2',3',5'-tri-O-acetyluridine (TAU); 1-[(2-hydroxyethoxy)methyl]-5-phenylthiouracil (PTAU); aryl-substituted 5-benzyluracils; 1-[(2-hydroxyethoxy)methyl]-5-benzyluracils; aryl-substituted 1-((2-hydroxyethoxy)methyl)-5-(3-phenoxybenzyl)uracil; 5-(benzyloxybenzyl)barbituric acid acyclonucleoside (BBBA); and hydroxymethyl-benzyloxybenzylacyclouridine (HMBBAU).

Additional suitable uridine phosphorylase inhibitors and methods for synthesizing such compounds are disclosed in the following references, all of which are hereby incorporated by reference: Chu et al., U.S. Pat. No. 4,613,604, Niedzwicki et al., 1981, Biochemical Pharmacology 30:2097-2101; Niedzwicki et al., 1982, Biochemical Pharmacology 31:1857-1861; and Lin et al., 1985, J. Med Chem. 25:971-973.

Derivatives of 5-benzyl barbiturate can also be used to inhibit uridine

phosphorylase. Such compounds, as are described in U.S. Pat. No. 5,141,943 (Naguib, et al.), include 5-benzyloxybenzyl barbiturate; 5-benzyloxybenzyl-1-[(1-hydroxy-2-ethoxy)methyl] barbiturate; 5-benzyloxybenzylacetyl-1-[(1-hydroxy-2-ethoxy)methyl] barbiturate; 5-benzyloxybenzyl-1-[(1,3-dihydroxy-2-propoxy)methyl] barbiturate; 5-benzyloxybenzyl-1-[1-hydroxy, 3-amino-2-propoxy)methyl] barbiturate; 5-benzyloxybenzyl-1-[(2-(3-carboxypropionyloxy)ethoxy)methyl] barbiturate; 5-benzyl-1-[(1-hydroxy-2-ethoxy)methyl] barbiturate; 5-methoxybenzylacetyl barbiturate; 5-benzyl-1-[(1, 3-dihydroxy-2-propoxy)methyl] barbiturate; 5-benzyl-1-[(1-hydroxy, 3-amino-2-propoxy)methyl] barbiturate; and 5-benzyl-1-[(2-(3-carboxypropionyloxy)ethoxy)methyl] barbiturate.

By the term "uridine phosphorylase inhibitor resistant" or "UPI-resistant" or "mutant" UPase, is meant a UPase that exhibits greater biological activity when contacted with a UPI than the activity demonstrated by wild type normal UPase when contacted with the same UPI under substantially indentical conditions. As disclosed elsewhere herein, biological activity of UPase can be assessed using a variety of methods including those disclosed herein, as well as methods known in the art or to be developed in the future.

By the term nucleic acid encoding "UPase resistant to a uridine phosphorylase inhibitor", "UPI-resistant UPase" or "mutant UPase", as used interchangeably herein, is meant any nucleic acid encoding a UPase protein wherein the nucleic acid has at least one mutation and where the UPase exhibits biological activity which is detectably greater than the UPase activity of a normal wild type UPase in the presence of a UPI.

Preferably, the UPase resistant to a UPI is encoded by a nucleic acid comprising a mutation, more preferably, the nucleic acid encoding UPase comprises at least one mutation in exon 1 and/or exon 6, where the genomic structure and organization of the UPase gene has been described by Cao et al. (1999, Cancer Res. 59:4997-5001). Even more preferably, the nucleic acid encoding the UPase resistant to a UPI comprises at least one of the following mutations: a G to A at nucleotide 40, a G to A at nucleotide

135, a G to C at nucleotide 178, a T to G at nucleotide 182, a G to A at nucleotide 186, a G to A at nucleotide 713, and a G to A at nucleotide 752.

The term "resistant", as used herein, means that the UPase is detectably less inhibited by a UPI, such as, for example, BAU, than the UPase activity of wild type UPase in the same concentration of the same UPI.

By the term "vector" as used herein, is meant any plasmid or virus encoding an exogenous nucleic acid. The term should also be construed to include non-plasmid and non-viral compounds which facilitate transfer of nucleic acid into virions or cells, such as, for example, polylysine compounds and the like. The vector may be a viral vector which is suitable as a delivery vehicle for delivery of the nucleic acid encoding UPase, or mutant thereof, to a cell, or the vector may be a non-viral vector which is suitable for the same purpose. Examples of viral and non-viral vectors for delivery of DNA to cells and tissues are well known in the art and are described, for example, in Ma et al. (1997, Proc. Natl. Acad. Sci. U.S.A. 94:12744-12746). Examples of viral vectors include, but are not limited to, a recombinant vaccinia virus, a recombinant adenovirus, a recombinant retrovirus, a recombinant adeno-associated virus, a recombinant avian pox virus, and the like (Cranage et al., 1986, EMBO J. 5:3057-3063; International Patent Application No. WO94/17810, published August 18, 1994; International Patent Application No. WO94/23744, published October 27, 1994). Examples of non-viral vectors include, but are not limited to, liposomes, polyamine derivatives of DNA, and the like.

By the terms "wild type" or "normal" UPase, as used interchangeably herein, is meant UPase that exhibits a typical level of inhibition when contacted with a uridine phosphorylase inhibitor. Preferably, the wild type UPase is encoded by a nucleic acid having the sequence SEQ ID NO:1. Alternatively, the wild type UPase has the amino acid sequence SEQ ID NO:2. More preferably, the wild type, normal UPase demonstrates the biological activity of UPase obtained from a normal tissue sample adjacent to a tumor tissue or from similar tissue obtained from a human known not to have a tumor in that tissue. Such tissues include, but are not limited to, breast, head-neck

and ovarian tissues, and the like.

Description

I. Isolated nucleic acids

A. Sense nucleic acids

The present invention includes a novel isolated nucleic acid encoding a human UPase where the UPase is resistant to a uridine phosphorylase inhibitor, such as, but not limited to, BAU. This is because applicants have discovered that the UPase present in certain tumor tissues is resistant to the inhibitory effects of UPIs such as BAU.

Further, the invention includes a novel isolated nucleic acid encoding a UPase resistant to a UPI where the nucleic acid comprises at least one mutation in exon 1 and/or exon 6. This is because applicants have discovered, using various techniques disclosed herein and/or those known in the art, that mutations in certain regions of the UPase gene are associated with or causally linked to resistance of the enzyme to inhibition by UPIs. Since the genomic structure and chromosomal location of UPase is known (Cao et al., 1999, Cancer Res. 59:4997-5001), one skilled in the art would appreciate, based upon the disclosure provided herein, that any mutation in a nucleic acid encoding UPase associated with resistance to UPIs can be readily mapped to the precise location on the UPase gene.

The present invention provides a novel isolated nucleic acid encoding a human UPase, wherein the nucleic acid has at least one mutation selected from the group consisting of a C to T at nucleotide 40, a C to T at nucleotide 135, a C to G at nucleotide 178, an A to C at nucleotide 182, a C to T at nucleotide 186, a G to A at nucleotide 713, and a G to A at nucleotide 752. Thus, the invention encompasses a nucleic acid comprising several, or all, mutations associated with increased resistance to the effect(s) of a UPI where the mutations are present in any combination or permutation of the above-disclosed mutations.

In addition to the nucleotide sequences of any nucleic acid encoding UPase having at least one of the mutations disclosed previously (*i.e.*, C to T at nucleotide

40, a C to T at nucleotide 135, a C to G at nucleotide 178, an A to C at nucleotide 182, a C to T at nucleotide 186, a G to A at nucleotide 713, and a G to A at nucleotide 752) of SEQ ID NO:1, it will be appreciated by those skilled in the art that DNA sequence polymorphisms that lead to changes in the amino acid sequence can exist within a population (*e.g.*, the human population) that are not associated with UPI resistance. Such genetic polymorphisms can exist among individuals within a population due to natural allelic variation. An allele is one of a group of genes which occur alternatively at a given genetic locus.

Accordingly, in another embodiment, an isolated *UPase* nucleic acid molecule of the invention encompasses a nucleic acid molecule comprising the nucleotide sequence, preferably the coding sequence, of SEQ ID NO:1, or a complement thereof, and comprising at least one of the mutations disclosed previously (*i.e.*, C to T at nucleotide 40, a C to T at nucleotide 135, a C to G at nucleotide 178, an A to C at nucleotide 182, a C to T at nucleotide 186, a G to A at nucleotide 713, and a G to A at nucleotide 752), further comprising additional mutations compared with SEQ ID NO:1, which mutations are not associated with UPI resistance. That is, the invention is not limited solely to a nucleic acid consisting solely of SEQ ID NO:1 with at least one of the above-disclosed mutations. Rather, the invention encompasses variants and mutants of normal wild type UPase (SEQ ID NO:1) such that the nucleic acid encoding such UPases comprise other mutations other than those disclosed herein but which mutations are not associated with UPI resistance.

One skilled in the art would understand, based upon the disclosure provided herein, that whether a mutation is associated with UPase resistance can be determined using the various methods disclosed herein. For example, a nucleic acid comprising the mutation of interest can be used to transfect a cell which is otherwise sensitive to UPI (*see, e.g.*, a cell transfected with rHUPase as disclosed elsewhere herein) and the resistance, if any, to UPI in such transfectant can be compared to control cells transfected with a nucleic acid not comprising the mutation of interest. Other assays, including enzymatic assays such as thin-layer chromatography (TLC)

based assays disclosed in Liu et al. (1998, Cancer Res. 58:5418-5424), and others known in the art or to be developed in the future, can also be used to assess the resistance of a UPase of interest, comprising a mutation, to one or more UPIs.

Additionally, the skilled artisan would appreciate, based on the disclosure provided herein, that a mutation of interest can be correlated with the enzyme kinetics of a UPase which is putatively resistant to a UPI to establish that the mutation is associated with resistance to a UPI. Such enzyme kinetics studies include those disclosed elsewhere herein, as well as others well-known in the art or to be developed in the future. Therefore, it would be appreciated by one skilled in the art, based upon the disclosure provided herein, that additional mutations associated with resistance to a uridine phosphorylase inhibitor can be readily identified and characterized following the teachings set forth herein and/or those well-known in the art. This is especially so since the UPase polypeptide is comprised of only about 310 amino acid residues and the coding region is comprised of approximately 1349 nucleotides such that it would be routine for the skilled artisan to identify and isolate additional mutations associated with UPI resistance.

The isolated nucleic acid of the invention should be construed to include an RNA or a DNA sequence encoding a mutant UPase protein of the invention, and any modified forms thereof, including chemical modifications of the DNA or RNA which render the nucleotide sequence more stable when it is cell free or when it is associated with a cell. Chemical modifications of nucleotides may also be used to enhance the efficiency with which a nucleotide sequence is taken up by a cell or the efficiency with which it is expressed in a cell. Any and all combinations of modifications of the nucleotide sequences are contemplated in the present invention.

One skilled in the art would appreciate, based upon the disclosure provided in the specification, that the invention includes a fragment of the nucleic acids of the invention. As used herein, the term "fragment" as applied to a nucleic acid, may ordinarily be at least about 20 nucleotides in length, typically, at least about 50 nucleotides, more typically, from about 100 to about 400 nucleotides, typically at least

about forty contiguous amino acids, preferably at least about 400 to about 800 nucleotides, even more preferably at least about 800 nucleotides to about 1200 nucleotides, more preferably, at least about 1200 nucleotides to about 1300, and more preferably, the nucleic acid fragment will be greater than about 1320 nucleotides in length.

The invention also includes a nucleic acid encoding UPase, and derivatives, variants, and fragments thereof, that can retain biological activity. Such variants, *i.e.,* analogs of UPase, include proteins or polypeptides which have been or may be modified using recombinant DNA technology such that the protein or polypeptide possesses additional properties which enhance its suitability for use in the methods described herein, for example, but not limited to, variants conferring enhanced stability on the UPase protein, and the like.

Procedures for the introduction of amino acid changes in a protein or polypeptide by altering the DNA sequence encoding the polypeptide are well known in the art and are also described in Sambrook et al. (1989, *supra*); Ausubel et al. (1997, *supra*).

The invention includes a nucleic acid encoding a UPase resistant to a UPI, including, but not limited to, SEQ ID NO:1 comprising at least one mutation selected from the group consisting of a C to T at nucleotide 40, a C to T at nucleotide 135, a C to G at nucleotide 178, an A to C at nucleotide 182, a C to T at nucleotide 186, a G to A at nucleotide 713, and a G to A at nucleotide 752, wherein a nucleic acid encoding a tag polypeptide is covalently linked thereto. That is, the invention encompasses a chimeric nucleic acid wherein a nucleic acid sequence encoding a tag polypeptide is covalently linked to a nucleic acid encoding a mutant UPI resistant UPase. Such chimeric (*i.e.,* fusion) tag polypeptides are well known in the art and include, for instance, myc, myc-pyruvate kinase (myc-PK), His_6, maltose biding protein (MBP), glutathione-S-transferase (GST), and green fluorescence protein (GFP). However, the invention is not limited to the nucleic acids encoding the above-listed tag polypeptides. Rather, any nucleic acid sequence encoding a polypeptide which may function in a manner substantially similar to

these tag polypeptides should be construed to be included in the present invention.

A nucleic acid encoding a protein of interest (*e.g.*, mutant UPase, and any mutant, derivative, variant, or fragment thereof) comprising a nucleic acid encoding a tag polypeptide and a fusion protein produced therefrom can be used to, among other things, localize mutant UPI-resistant UPase within a cell and to study expression, localization, and role(s) of the tagged protein in a cell before, during, and/or after exposing the cell to a test compound. Further, addition of a tag to a protein of interest facilitates isolation and purification of the "tagged" protein such that the protein of interest can be easily produced and purified.

In other related aspects, the invention includes a nucleic acid encoding a UPI-resistant, *i.e.*, mutant, *UPase* operably linked to a nucleic acid comprising a promoter/regulatory sequence such that the nucleic acid is preferably capable of directing expression of the protein encoded by the nucleic acid.

Expression of UPI-resistant UPase either alone or fused to a detectable tag polypeptide in cells which either do not normally express UPI resistant UPase or which do not express UPI resistant UPase comprising a tag polypeptide, can be accomplished by operably linking the nucleic acid encoding UPI resistant UPase to a promoter/regulatory sequence which serves to drive expression of the protein, with or without a tag polypeptide, in cells in which the exogenous nucleic acid (*i.e.*, transgene) is introduced.

Many promoter/regulatory sequences useful for driving constitutive expression of a gene are available in the art and include, but are not limited to, for example, the cytomegalovirus immediate early promoter enhancer sequence, the SV40 early promoter, both of which were used in the experiments disclosed herein, as well as the Rous sarcoma virus promoter, and the like. Moreover, inducible and tissue specific expression of the nucleic acid encoding UPI resistant *UPase* may be accomplished by placing the nucleic acid encoding UPI resistant *UPase*, with or without a tag polypeptide, under the control of an inducible or tissue specific promoter/regulatory sequence. Examples of tissue specific or inducible promoter/regulatory sequences which are useful for his purpose include, but are not limited to the MMTV LTR inducible promoter, and

the SV40 late enhancer/promoter. In addition, promoters which are well known in the art which are induced in response to inducing agents such as metals, glucocorticoids, and the like, are also contemplated in the invention. Thus, it will be appreciated that the invention includes the use of any promoter/regulatory sequence, which is either known or unknown, and which is capable of driving expression of the desired protein encoded by a nucleic acid operably linked to the promoter/regulatory sequence.

Expressing UPI-resistant UPase using a promoter/regulatory sequence allows the isolation of large amounts of recombinantly produced protein. Further, where the decreased or lack of expression of UPI-resistant UPase is associated with and/or mediates a disease, disorder, or condition associated with such expression, the expression of the protein driven by a promoter/regulatory sequence can provide useful therapeutics including, but not limited to, gene therapy whereby the protein is provided. This is because administering UPI-resistant UPase to a cell, whether by administering a nucleic acid encoding the UPase or by providing the protein itself to a cell, can render the cell more susceptible to the toxic effects of certain chemotherapeutic agents, *e.g.*, AZT and 5-FU, such that by providing the UPI-resistant UPase to certain target cells renders such cells more sensitive to the toxic effects of the agents relative to non-target cells that do not express UPI-resistant UPase and where uridine levels are preserved by the UPase inhibitory effects of a UPI that is co-administered with the chemotherapeutic agent.

B. Antisense nucleic acids

In certain situations, it may be desirable to inhibit expression of UPI-resistant UPase in a cell that would otherwise express the protein. For example, the invention encompasses, but is not limited to, inhibiting mutant UPase expression where over-expression (*e.g.*, expression at a level higher than the normal level) of mutant UPase mediates a disease, disorder or condition, such as, but not limited to, breast, head-neck and ovarian cancer, and the like. Further, inhibiting expression of UPI-resistant UPase a cell provides an *in vitro* model system for the study of a disease, disorder or condition mediated by expression of UPI-resistant UPase. Such a cell can provide a useful *in vitro*

model system for the study of, for instance, the mechanism of such disease, disorder or condition, including the role, if any, of expression of UPI-resistant UPase in such disease, disorder or condition, as well as for the identification of therapeutic treatments therefor. That is, a cell that does not express UPase wherein an otherwise identical cell does express the protein, is an important model for the study of the role(s) of UPase in a cell, including the role(s) of UPase in tumorgenesis.

Therefore, the invention includes compositions useful for inhibition of expression of wild type and/or mutant UPase (*e.g.*, UPase comprising a mutation which renders the enzyme resistant to inhibition by BAU, and the like) and isoforms thereof. One such method of the invention features an isolated nucleic acid complementary to a portion or all of a nucleic acid encoding a UPase (mutant or wild type) which is in an antisense orientation with respect to transcription. Preferably, the isolated antisense nucleic acid useful for inhibition of UPase expression hybridizes with a nucleic acid encoding human UPase, more preferably, the nucleic acid comprises at least one mutation (*e.g.*, C to T at nucleotide 40, a C to T at nucleotide 135, a C to G at nucleotide 178, an A to C at nucleotide 182, a C to T at nucleotide 186, a G to A at nucleotide 713, and a G to A at nucleotide 752), or a portion thereof.

The above-referred to antisense nucleic acids serve to inhibit the expression, function, or both, of wild type UPase and/or mutant UPase resistant to UPIs, including BAU.

Additionally, the use of ribozymes to effect an inhibition of expression of UPase is contemplated in the present invention, as is the use of any other means which would effect a reduction in expression of UPase. Means of inhibiting expression of a desired nucleic acid encoding a protein of interest are well-known in the art and are described, for example, in Sambrook et al. (1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York) and Ausubel et al. (1997, Current Protocols in Molecular Biology, Green & Wiley, New York).

One skilled in the art will appreciate, based on the disclosure provided herein, that the level of mutant UPase protein in a cell can be decreased by reducing or

inhibiting expression of the *UPase* gene. Thus, the level of mutant UPase protein in a cell can be decreased using a molecule or compound that inhibits or reduces gene expression such as, for example, antisense molecules, ribozymes, or double-stranded RNA as described in, for example, Wianny and Kernicka-Goetz (2000, Nature Cell Biol. 2:70-75).

In a one embodiment, the mutant UPase modulating sequence is a mutant *UPase* antisense nucleic acid sequence which is encoded by a plasmid vector and which is used to transfect a mammalian cell thereby causing reduced endogenous expression of mutant UPase protein in the cells. However, as stated previously herein, the invention should not be construed to be limited to inhibiting expression of mutant UPase by transfection of cells with antisense molecules. Rather, the invention encompasses other methods known in the art for inhibiting expression of a protein in cells including, but not limited to, the use of ribozymes, double stranded RNA, and expression of a non-functional UPase under the control of, for example, an inducible promoter, and the like.

With regard to using antisense molecules to inhibit gene expression, such use is well known in the art (*see, e.g.,* Cohen, 1989, In: Oligodeoxyribonucleotides, Antisense Inhibitors of Gene Expression, CRC Press). Antisense nucleic acids are DNA or RNA molecules that are complementary, as that term is defined elsewhere herein, to at least a portion of a specific mRNA molecule (Weintraub, 1990, Scientific American 262:40). In the cell, antisense nucleic acids hybridize to the corresponding mRNA, forming a double-stranded molecule thereby inhibiting the translation of genes.

The use of antisense methods to inhibit the translation of genes is known in the art, and is described, for example, in Marcus-Sakura (1988, Anal. Biochem. 172:289). Such antisense molecules may be provided to the cell via genetic expression using DNA encoding the antisense molecule as taught by Inoue, 1993, U.S. Patent No. 5,190,931.

Alternatively, antisense molecules of the invention may be made synthetically and then provided to the cell. Antisense oligomers of between about 10 to about 30, and more preferably about 15 nucleotides, are preferred, since they are easily synthesized and introduced into a target cell. Synthetic antisense molecules contemplated

by the invention include oligonucleotide derivatives known in the art which have improved biological activity compared to unmodified oligonucleotides (*see* Cohen, *supra*; Tullis, 1991, U.S. Patent No. 5,023,243, incorporated by reference herein in its entirety).

Ribozymes and their use for inhibiting gene expression are also well known in the art (*see, e.g.,* Cech et al., 1992, J. Biol. Chem. 267:17479-17482; Hampel et al., 1989, Biochemistry 28:4929-4933; Eckstein et al., International Publication No. WO 92/07065; Altman et al., U.S. Patent No. 5,168,053, incorporated by reference herein in its entirety). Ribozymes are RNA molecules possessing the ability to specifically cleave other single-stranded RNA in a manner analogous to DNA restriction endonucleases. Through the modification of nucleotide sequences encoding these RNAs, molecules can be engineered to recognize specific nucleotide sequences in an RNA molecule and cleave it (Cech, 1988, J. Amer. Med. Assn. 260:3030). A major advantage of this approach is that, because they are sequence-specific, only mRNAs with particular sequences are inactivated.

There are two basic types of ribozymes, namely, tetrahymena-type (Hasselhoff, 1988, Nature 334:585) and hammerhead-type. Tetrahymena-type ribozymes recognize sequences which are four bases in length, while hammerhead-type ribozymes recognize base sequences 11-18 bases in length. The longer the sequence, the greater the likelihood that the sequence will occur exclusively in the target mRNA species. Consequently, hammerhead-type ribozymes are preferable to tetrahymena-type ribozymes for inactivating specific mRNA species, and 18-base recognition sequences are preferable to shorter recognition sequences which may occur randomly within various unrelated mRNA molecules.

Ribozymes useful for inhibiting the expression of mutant UPase may be designed by incorporating target sequences into the basic ribozyme structure which are complementary to the mRNA sequence of the a nucleic acid encoding UPI-resistant UPase. Ribozymes targeting mutant *UPase* can be synthesized using commercially available reagents (Applied Biosystems, Inc., Foster City, CA) or they may be genetically expressed from DNA encoding them.

One skilled in the art would understand, based upon the disclosure provided herein, that the present invention encompasses methods of inhibiting expression of both wild type and mutant UPase. That is, antisense nucleic acids and ribozymes can be designed to selectively inhibit wild type UPase, mutant UPase, or both.

5

II. Vectors

The invention also includes a vector comprising a nucleic acid encoding a mutant UPase. Methods for incorporating a desired nucleic acid into a vector and the choice of vectors is well-known in the art as described in, for example, Sambrook et al., 10 *supra*, and Ausubel et al., *supra*.

Further, the invention encompasses expression vectors and methods for the introduction of exogenous nucleic acid encoding mutant *UPase* into a cell with concomitant expression of the exogenous nucleic acid in the cell using such methods as those described in, for example, Sambrook et al. (1989, *supra*), and Ausubel et al. (1997, 15 *supra*), and as disclosed elsewhere herein.

Selection of any particular plasmid vector or other DNA vector is not a limiting factor in this invention and a wide plethora vectors are well-known in the art (*see, e.g.*, Sambrook et al., *supra*, and Ausubel et al., *supra*.). Further, it is well within the skill of the artisan to choose particular promoter/regulatory sequences and operably 20 link those promoter/regulatory sequences to a DNA sequence encoding a desired polypeptide. Such technology is well known in the art and is described, for example, in Sambrook, *supra*, and Ausubel, *supra*.

The invention includes also cells, viruses, proviruses, and the like, containing such vectors. Methods for producing cells comprising vectors and/or 25 exogenous nucleic acids are well-known in the art. *See, e.g.*, Sambrook et al., *supra*; Ausubel et al., *supra*.

The nucleic acids encoding mutant *UPase* can be cloned into various plasmid vectors. However, the present invention should not be construed to be limited to plasmids or to any particular vector. Instead, the present invention should be construed to

encompass a wide plethora of vectors which are readily available and/or well-known in the art.

III. Recombinant Cells

Additionally, the nucleic and amino acids of the invention can be used to produce recombinant cells which are useful tools for the study of UPase activity and resistance to UPIs such as, but not limited to, BAU, for the identification of novel therapeutics, and for elucidating the cellular role(s) of mutant UPase, among other things.

Further, the nucleic and amino acids of the invention can be used diagnostically, either by assessing the level of gene expression or protein expression and the biological activity of the protein, and to detect and/or assess severity and prognosis of a disease, disorder, or condition associated with expression of UPI-resistant UPase where the enzyme is resistant to BAU, among other inhibitors.

The invention also includes expression of human UPI-resistant UPase in a cell where it is not normally expressed or expression of UPI-resistant UPase-tagged fusion protein in cells where this fusion protein is not normally expressed. In a preferred embodiment, nucleic acid encoding human UPI-resistant UPase can be tagged with a nucleic acid expressing a tag polypeptide to transfect a mammalian cell. Plasmid constructs containing UPI-resistant UPase, or mutants, variants, derivatives and fragments thereof, can be cloned into a wide variety of vectors including a vector comprising a nucleic acid encoding a tag polypeptide. The plasmids can be introduced into a cell using standard methods well-known in the art (*e.g.*, calcium phosphate, electroporation, and the like). Methods for cloning and introducing an isolated nucleic acid of interest into a cell are exemplified herein and are described in, for example, Sambrook et al. (1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York), Ausubel et al. (1997, Current Protocols in Molecular Biology, Green & Wiley, New York), and other standard treatises.

The present invention also encompasses expression of an isolated UPI-resistant UPase of the invention in amphibian and other non-mammalian cells (*e.g.* yeast,

insect, and avian cells) using methods well-known in the art such as those disclosed elsewhere herein. Thus, it is clear that the invention is not limited to any particular vector or to any particular method of introducing the exogenous nucleic acid encoding UPI-resistant UPase into a cell.

Expression of proteins of interest (*e.g.*, UPI-resistant Upase and isoforms thereof) in a cell, especially when the protein comprises a tag polypeptide, allows localization of the nucleic acid and/or the protein expressed therefrom within the cell under selected conditions such that the function(s) of the protein in the cell can be studied and identified.

One skilled in the art would appreciate, based upon the disclosure provided herein, that the invention also includes expression of UPI-resistant UPase, and the like, in prokaryotic cells (*e.g.*, bacterial cells such as, for example, *E. coli*). Accordingly, the invention includes expression of the proteins of the invention in such cells as well.

The invention should not be construed as being limited to these plasmid vectors, bacterial strains, or to these tag polypeptides. Further, the invention is not limited to any particular method of introducing a nucleic acid into a cell or any particular cell type or as exemplified herein. Instead, the invention encompasses other expression vectors and methods for the introduction of exogenous DNA into cells with concomitant expression of the exogenous DNA in the cells such as those described, for example, in Sambrook et al. (1989, *supra*), and Ausubel et al. (1997, *supra*).

In one embodiment, nucleic acids encoding various UPases were introduced into bacterial cells using electroporation. The nucleic acid were cloned into a plasmid expression vector (pQE) expressed under the control of a promoter/regulatory sequence. The cells can be transfected with constructs which comprise UPI-resistant UPase cDNA in either a sense (*i.e.*, sense cells) or an antisense orientation (*i.e.*, antisense cells).

One skilled in the art would further appreciate that selected forms of nucleic acids encoding *UPase* can be introduced to a cell in order to study the effect of any mutant, derivative, and variant of Upase (*e.g.*, fusion proteins comprising at least a

portion of UPase, including UPI-resistant UPase, and a tag polypeptide) in this system.

Further, the invention includes a recombinant cell comprising an antisense nucleic acid which cell is a useful model for the study of a disease, disorder, or condition associated with or mediated by inhibition of UPI-resistant UPase biosynthesis and for elucidating the role(s) of UPI-resistant UPase in such processes. That is, the lack of expression of UPI-resistant UPase in a human may treat and/or alleviate, among other things, a disease, disorder or condition. Accordingly, a recombinant (*i.e.*, transgenic) cell comprising an antisense nucleic acid complementary to *UPI-resistant UPase* is a useful tool for the study of the mechanism(s) of action of UPI-resistant UPase and its potential role(s) in the cell, and in carcinogenesis, tumorgenesis, and cell differentiation, as well as for the identification of therapeutics to treat certain tumors (*e.g.*, breast, head-neck, and ovarian tumors, and the like). This is because one skilled in the art would understand, based upon the disclosure provided herein, that the data disclosed herein indicate that a mutation in UPase associated with UPI resistance is associated with and/or may mediate the carcinogenesis process. Thus, such mutation is associated with tumor development and is a useful diagnostic and potential therapeutic target therefor.

The invention further includes a recombinant cell comprising an isolated nucleic acid encoding UPI-resistant UPase. The cell can be transiently transfected with a plasmid encoding a portion of the nucleic acid encoding the protein of interest, *e.g.*, *UPase*. The nucleic acid need not be integrated into the cell genome nor does it need to be expressed in the cell. Moreover, the cell may be a prokaryotic or a eukaryotic cell and the invention should not be construed to be limited to any particular cell line or cell type.

When the cell is a eukaryotic cell, the cell may be any eukaryotic cell which, when the isolated nucleic acid of the invention is introduced therein, and the protein encoded by the desired gene is no longer expressed therefrom, a benefit is obtained. Such a benefit may include the fact that there has been provided a system in which lack of expression of the desired gene can be studied *in vitro* in the laboratory or in a mammal in which the cell resides, a system wherein cells comprising the introduced gene deletion can be used as research, diagnostic and therapeutic tools, and a system

wherein animal models are generated which are useful for the development of new diagnostic and therapeutic tools for selected disease, disorder, or condition states in a mammal, including, but not limited to, tumors, *e.g.*, breast, head-neck, and ovarian tumors, and the like.

Alternatively, the invention includes a eukaryotic cell which, when the isolated nucleic acid of the invention is introduced therein, and the protein encoded by the desired gene, *i.e.*, UPI-resistant UPase, is expressed therefrom where it was not previously present or expressed in the cell or where it is now expressed at a level or under circumstances different than that before the isolated nucleic acid was introduced, a benefit is obtained. Such a benefit may include the fact that there has been provided a system wherein the expression of the desired gene can be studied *in vitro* in the laboratory or in a mammal in which the cell resides, a system wherein cells comprising the introduced gene can be used as research, diagnostic and therapeutic tools, and a system wherein animal models are generated which are useful for the development of new diagnostic and therapeutic tools for selected disease states in a mammal (*e.g.*, diseases, disorders or conditions relating to tumors of breast, head-neck and ovarian tissues associated with or mediated by expression of UPI-resistant UPase).

IV. Isolated polypeptides

The invention also includes a novel isolated polypeptide encoded by a nucleic acid encoding a human UPI-resistant UPase. Preferably, the nucleic acid comprises at least one mutation in exon 1 and/or exon 6 which mutation is associated with or mediates UPI resistance in the UPase.

More preferably, the isolated nucleic acid encoding the human UPI-resistant UPase comprises at least one mutation selected from the following mutations: C to T at nucleotide 40, a C to T at nucleotide 135, a C to G at nucleotide 178, an A to C at nucleotide 182, a C to T at nucleotide 186, a G to A at nucleotide 713, and a G to A at nucleotide 752. The skilled artisan would appreciate, based upon the disclosure provided herein, that the mutations can occur in any combination and/or permutation and the

invention is in no way limited to any particular mutation or combination thereof.

The invention also includes an isolated human uridine phosphorylase polypeptide wherein the polypeptide is resistant to UPI compared with wild type normal UPase (*e.g.*, that described by Watanabe et al., 1995, Biochem. Biophys. Res. Commun. 216:265-272, *i.e.*, SEQ ID NO:2) and where the polypeptide comprises at least one mutation selected from a change from glutamic acid to lysine at amino acid residue number 121, and a change from valine to isoleucine at amino acid residue number 134.

One skilled in the art would appreciate, based on the disclosure provided herein, that the UPI-resistant UPase of the invention can comprise more than on mutation associated with UPI resistance. Further, as more fully set forth below, the invention encompasses UPI-resistant UPase comprising other mutations that are not associated with UPI resistance as well as mutations that are so associated. This is because the UPase of the invention can comprise mutations occurring at non-essential positions in the polypeptide molecule, conservative amino acid substitutions, and mutations located at amino acids not involved in enzyme activity (*i.e.*, amino acids that are not located in the putative UPase catalytic site) or which do not alter the requisite protein conformation can be present in a UPase and not affect the UPI resistance of the enzyme. Such mutations are encompassed in the invention such that the UPase of the invention is not limited to having solely the sequence SEQ ID NO:2 except for having mutations associated with UPI resistance; rather, the invention encompasses UPI resistant UPase comprising additional mutations, besides those mediating UPI resistance, which additional mutations do not affect biological activity of UPase.

The present invention also provides for analogs of proteins or peptides which comprise a human UPI-resistant UPase protein as disclosed herein. Analogs may differ from naturally occurring proteins or peptides by conservative amino acid sequence differences or by modifications which do not affect sequence, or by both. For example, conservative amino acid changes may be made, which although they alter the primary sequence of the protein or peptide, do not normally alter its function. Conservative amino acid substitutions typically include substitutions within the following groups:

glycine, alanine;
valine, isoleucine, leucine;
aspartic acid, glutamic acid;
asparagine, glutamine;
serine, threonine;
lysine, arginine;
phenylalanine, tyrosine;

Modifications (which do not normally alter primary sequence) include *in vivo*, or *in vitro*, chemical derivatization of polypeptides, *e.g.*, acetylation, or carboxylation. Also included are modifications of glycosylation, *e.g.*, those made by modifying the glycosylation patterns of a polypeptide during its synthesis and processing or in further processing steps; *e.g.*, by exposing the polypeptide to enzymes which affect glycosylation, *e.g.*, mammalian glycosylating or deglycosylating enzymes. Also embraced are sequences which have phosphorylated amino acid residues, *e.g.*, phosphotyrosine, phosphoserine, or phosphothreonine.

Also included are polypeptides which have been modified using ordinary molecular biological techniques so as to improve their resistance to proteolytic degradation or to optimize solubility properties or to render them more suitable as a therapeutic agent. Analogs of such polypeptides include those containing residues other than naturally occurring L-amino acids, *e.g.*, D-amino acids or non-naturally occurring synthetic amino acids. The peptides of the invention are not limited to products of any of the specific exemplary processes listed herein.

The present invention should also be construed to encompass "mutants," "derivatives," and "variants" of the peptides of the invention (or of the DNA encoding the same) which mutants, derivatives and variants are UPI-resistant UPase peptides which are altered in one or more amino acids (or, when referring to the nucleotide sequence encoding the same, are altered in one or more base pairs) such that the resulting peptide (or DNA) is not identical to the sequences recited herein, but has the same biological

property as the peptides disclosed herein, in that the peptide has biological/biochemical properties of the UPI-resistant UPase peptide of the present invention. A biological property of a UPI-resistant UPase includes, but is not limited to include, the ability of the protein to catalyze the phosphorylization of uridine and, to a lesser extent, thymidine, similar to the wild type UPase protein (SEQ ID NO:2). Further, the UPI-resistant UPase of the invention is resistant to the inhibitory effects of UPIs compared with the sensitivity of wild type UPase to such compounds.

Further, the invention should be construed to include naturally occurring variants or recombinantly derived mutants of UPI-resistant UPase, which variants or mutants render the protein encoded thereby either more, less, or just as biologically active as the full-length proteins of the invention.

In addition to naturally-occurring allelic variants of a peptide molecule of the invention, the skilled artisan will further appreciate that changes can be introduced by mutation of the nucleic acid encoding the protein leading to changes in the amino acid sequence of the encoded protein, without altering the biological activity of the protein. For example, one can make nucleotide substitutions leading to amino acid substitutions at "non-essential" amino acid residues. A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence without altering the biological activity, whereas an "essential" amino acid residue is required for biological activity. For example, amino acid residues that are not conserved or only semi-conserved among homologs of various species may be non-essential for activity and thus would be likely targets for alteration. Alternatively, amino acid residues that are conserved among the homologs of various species (*e.g.*, murine and human) may be essential for activity and thus would not be likely targets for alteration.

Accordingly, another aspect of the invention pertains to polypeptides encoded by nucleic acid molecules of the invention, which polypeptides contain changes in amino acid residues that are not essential for activity. The skilled artisan would appreciate, based upon the disclosure provided herein, that amino acid changes located in amino acids that do not comprise the putative UPase catalytic site (*i.e.*,

amino acids from about residue number 107 through 122), are less likely to affect the biological function of the protein than changes in amino acids that form part of the active site.

To generate variant proteins, an isolated nucleic acid molecule encoding a variant protein can be created by introducing one or more nucleotide substitutions, additions or deletions into a wild type nucleotide sequence such as, but not limited to, SEQ ID NO:1, such that one or more amino acid residue substitutions, additions or deletions are introduced into the encoded UPase protein. Mutations can be introduced by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis.

Preferably, conservative amino acid substitutions are made at one or more predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), non-polar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Alternatively, mutations can be introduced randomly along all or part of the coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for biological activity to identify mutants that retain activity. Following mutagenesis, the encoded protein can be expressed recombinantly and the activity of the protein can be determined.

One skilled in the art would appreciate, based upon the disclosure provided herein, that a mutant polypeptide that is a variant of a polypeptide of the invention can be assayed for: (1) the ability to phosphorylize uridine and, to a lesser extent, thymidine and (2) decreased inhibition in activity when contacted with a UPI

such as, but not limited to, BAU.

The nucleic acids, and peptides encoded thereby, are useful tools for elucidating the function(s) of UPI-resistant UPase in a cell. Further, they are useful for localizing the nucleic acid, protein, or both, in a cell and for assessing the level of expression of the nucleic acid and/or protein under selected conditions including in response to therapeutic treatment. Further, nucleic and amino acids comprising human UPI-resistant UPase useful diagnostics which can be used, for example, to identify a human afflicted with a disease, disorder, or condition associated with expression of UPI-resistant UPase.

In addition, the nucleic acids, the proteins encoded thereby, or both, can be administered to a human to increase or decrease expression of UPI-resistant UPase in the human. This can be therapeutic to the human if lack of, or expression of, UPI-resistant UPase in the human mediates a disease or condition associated with expression of the protein compared with expression of normal, wild type UPase in an otherwise identical healthy human.

V. Antibodies

The invention also includes an antibody specific for a human UPI resistant UPase, or a portion thereof.

In one embodiment, the antibody is a rabbit polyclonal antibody to UPI-resistant UPase. The antibody can be specific for any portion of the protein and the full-length protein can be used to generate antibodies specific therefor. However, the present invention is not limited to using the full-length protein as an immunogen. Rather, the present invention includes using an immunogenic portion of the protein to produce an antibody that specifically binds with UPI-resistant UPase but not with wild type UPase. That is, the invention includes immunizing an animal using an immunogenic portion comprising, for example, amino acid residues (*i.e.*, the sequence comprising the putative catalytic site comprising amino acids from about residue number 107 through 122) or a portion comprising the mutations associated with UPI resistance, *e.g.*, change from

glutamic acid to lysine at amino acid residue 121, and valine to isoleucine at amino acid residue number 134, or a combination of these portions. Preferably, the immunogenic portion is a fragment of the UPI-resistant UPase comprising from about amino acid residue 116 to about amino acid residue 124 (*i.e.*, comprising the mutation at amino acid 121), and a fragment comprising from about amino acid residue 131 to about amino acid residue 139 (*i.e.*, comprising the mutation at amino acid 134). It would be understood by one skilled in the art, based upon the disclosure provided herein, that the present invention is not limited to any particular portion of UPI-resistant UPase; rather, the invention encompasses production of an antibody that specifically binds with UPI-resistant UPase but not with wild type UPase regardless of how the antibody is produced. This is because one skilled in the art would appreciate that production of antibodies with such specificity is routine in the art such one could readily produce those antibodies once armed with the teachings of the present invention that UPase in certain cells is resistant to UPIs and that such resistance is associated with mutations in the UPase gene.

The antibodies can be produced by immunizing an animal such as, but not limited to, a rabbit or a mouse with a protein of the invention, or a portion thereof, or by immunizing an animal using a protein comprising at least a portion of UPI-resistant UPase and a tag polypeptide portion comprising, for example, a maltose binding protein tag polypeptide portion and a portion comprising the respective UPI-resistance UPase amino acid residues. Further, antibodies can be produced using UPI-resistant UPase isolated using BAU-affinity chromatography. Such methods are described in Liu et al., 1998, Cancer Res. 58:5418-5424, which is incorporated herein by reference.

One skilled in the art would appreciate, based upon the disclosure provided herein, that various portions of an isolated UPI-resistant UPase polypeptide can be used to generate antibodies to either highly conserved regions of UPase (*e.g.*, the phosphorylase family 1 signature pattern as described in Liu et al., 1998, Cancer Res. 58:5418-5424 at Table 2) or to non-conserved regions such as those disclosed previously, or both. The skilled artisan, based upon the disclosure provided herein, would appreciate that the non-conserved regions of a protein of interest can be more immunogenic than the highly

conserved regions which are conserved among various organisms. Further, immunization using a non-conserved immunogenic portion can produce antibodies specific for the non-conserved region thereby producing antibodies that do not cross-react with other proteins which can share one or more conserved portions. Thus, by comparing the amino acid sequences of wild type and UPI-resistant UPase, the skilled artisan can determine, based upon the disclosure provided herein, proper portions of UPase to use as immunogen to generate UPI-resistant specific antibodies, cross-reacting antibodies recognizing both wild type and resistant UPase, or both.

The invention encompasses polyclonal, monoclonal, synthetic antibodies, and the like. One skilled in the art would understand, based upon the disclosure provided herein, that the crucial feature of the antibody of the invention is that the antibody bind specifically with UPI-resistant UPase. That is, the antibody of the invention recognizes UPI-resistant UPase, or a fragment thereof, on Western blots, in immunostaining of cells, and immunoprecipitates UPI-resistant UPase using standard methods well-known in the art.

One skilled in the art would appreciate, based upon the disclosure provided herein, that the antibodies can be used to localize the relevant protein in a cell and to study the role(s) of the antigen recognized thereby in cell processes. Moreover, the antibodies can be used to detect and or measure the amount of protein present in a biological sample using well-known methods such as, but not limited to, Western blotting and enzyme-linked immunosorbent assay (ELISA). Moreover, the antibodies can be used to immunoprecipitate and/or immuno-affinity purify their cognate antigen using methods well-known in the art.

The generation of polyclonal antibodies is accomplished by inoculating the desired animal with the antigen and isolating antibodies which specifically bind the antigen therefrom using standard antibody production methods such as those described in, for example, Harlow et al. (1988, In: Antibodies, A Laboratory Manual, Cold Spring Harbor, NY).

Monoclonal antibodies directed against full length or peptide fragments of

a protein or peptide can be prepared using any well known monoclonal antibody preparation procedures, such as those described, for example, in Harlow et al., 1988, *supra*, and in Tuszynski et al. (1988, Blood, 72:109-115), and methods set forth elsewhere herein. Quantities of the desired peptide may also be synthesized using chemical synthesis technology. Alternatively, DNA encoding the desired peptide may be cloned and expressed from an appropriate promoter sequence in cells suitable for the generation of large quantities of peptide. Monoclonal antibodies directed against the peptide are generated from mice immunized with the peptide using standard procedures as referenced herein.

Nucleic acid encoding the monoclonal antibody obtained using the procedures described herein may be cloned and sequenced using technology which is available in the art, and is described, for example, in Wright et al. (1992, Critical Rev. Immunol. 12:125-168), and the references cited therein. Further, the antibody of the invention may be "humanized" using the technology described in Wright et al. (*supra*), and in the references cited therein, and in Gu et al. (1997, Thrombosis and Hematocyst 77:755-759).

To generate a phage antibody library, a cDNA library is first obtained from mRNA which is isolated from cells, *e.g.*, the hybridoma, which express the desired protein to be expressed on the phage surface, *e.g.*, the desired antibody. cDNA copies of the mRNA are produced using reverse transcriptase. cDNA which specifies immunoglobulin fragments are obtained by PCR and the resulting DNA is cloned into a suitable bacteriophage vector to generate a bacteriophage DNA library comprising DNA specifying immunoglobulin genes. The procedures for making a bacteriophage library comprising heterologous DNA are well known in the art and are described, for example, in Sambrook et al., *supra*.

Bacteriophage which encode the desired antibody, may be engineered such that the protein is displayed on the surface thereof in such a manner that it is available for binding to its corresponding binding protein, *e.g.*, the antigen against which the antibody is directed. Thus, when bacteriophage which express a specific antibody are incubated in

the presence of a cell which expresses the corresponding antigen, the bacteriophage will bind to the cell. Bacteriophage which do not express the antibody will not bind to the cell. Such panning techniques are well known in the art and are described for example, in Wright et al. (*supra*).

Processes such as those described above, have been developed for the production of human antibodies using M13 bacteriophage display (Burton et al., 1994, Adv. Immunol. 57:191-280). Essentially, a cDNA library is generated from mRNA obtained from a population of antibody-producing cells. The mRNA encodes rearranged immunoglobulin genes and thus, the cDNA encodes the same. Amplified cDNA is cloned into M13 expression vectors creating a library of phage which express human Fab fragments on their surface. Phage which display the antibody of interest are selected by antigen binding and are propagated in bacteria to produce soluble human Fab immunoglobulin. Thus, in contrast to conventional monoclonal antibody synthesis, this procedure immortalizes DNA encoding human immunoglobulin rather than cells which express human immunoglobulin.

The procedures just presented describe the generation of phage which encode the Fab portion of an antibody molecule. However, the invention should not be construed to be limited solely to the generation of phage encoding Fab antibodies. Rather, phage which encode single chain antibodies (scFv/phage antibody libraries) are also included in the invention. Fab molecules comprise the entire Ig light chain, that is, they comprise both the variable and constant region of the light chain, but include only the variable region and first constant region domain (CH1) of the heavy chain. Single chain antibody molecules comprise a single chain of protein comprising the Ig Fv fragment. An Ig Fv fragment includes only the variable regions of the heavy and light chains of the antibody, having no constant region contained therein. Phage libraries comprising scFv DNA may be generated following the procedures described in Marks et al. (1991, J. Mol. Biol. 222:581-597). Panning of phage so generated for the isolation of a desired antibody is conducted in a manner similar to that described for phage libraries comprising Fab DNA.

The invention should also be construed to include synthetic phage display libraries in which the heavy and light chain variable regions may be synthesized such that they include nearly all possible specificities (Barbas, 1995, Nature Medicine 1:837-839; de Kruif et al. 1995, J. Mol. Biol. 248:97-105).

VI. Transgenic non-human mammals

As disclosed previously elsewhere herein, the invention includes a recombinant cell comprising , *inter alia*, an isolated nucleic acid encoding UPI-resistant UPase, an antisense nucleic acid complementary thereto, and the like.

In one aspect, the recombinant cell comprising an isolated nucleic acid encoding human UPI-resistant UPase is used to produce a transgenic non-human mammal. That is, the exogenous nucleic acid, or "transgene" as it is also referred to herein, of the invention is introduced into a cell, and the cell is then used to generate the non-human transgenic mammal.

The cell into which the transgene is introduced is preferably an embryonic stem (ES) cell. However, the invention should not be construed to be limited solely to ES cells comprising the transgene of the invention nor to cells used to produce transgenic animals. Rather, a transgenic cell of the invention includes, but is not limited to, any cell derived from a transgenic animal comprising a transgene, a cell comprising the transgene derived from a chimeric animal derived from the transgenic ES cell, and any other cell comprising the transgene, which cell may or may not be used to generate a non-human transgenic mammal.

Further, it is important to note that the purpose of recombinant, *i.e.*, transgene-comprising, cells should not be construed to be limited to the generation of transgenic mammals. Rather, the invention should be construed to include any cell type into which a nucleic acid encoding a human UPI-resistant UPase is introduced, including, without limitation, a prokaryotic cell and a eukaryotic cell comprising an isolated nucleic acid encoding UPI-resistant UPase.

When the cell is a eukaryotic cell, the cell may be any eukaryotic cell

which, when the transgene of the invention is introduced therein, and the protein encoded by the desired gene is no longer expressed therefrom, a benefit is obtained. Such a benefit may include the fact that there has been provided a system in which lack of expression of the desired gene can be studied *in vitro* in the laboratory or in a mammal in which the cell resides, a system wherein cells comprising the introduced gene deletion can be used as research, diagnostic and therapeutic tools, and a system wherein animal models are generated which are useful for the development of new diagnostic and therapeutic tools for selected disease states in a mammal including, for example, breast tumors, head-neck tumors, ovarian tumors, and the like.

Alternatively, the invention includes a eukaryotic cell which, when the transgene of the invention is introduced therein, and the protein encoded by the desired gene is expressed therefrom where it was not previously present or expressed in the cell or where it is now expressed at a level or under circumstances different than that before the transgene was introduced, a benefit is obtained. Such a benefit may include the fact that there has been provided a system in the expression of the desired gene can be studied *in vitro* in the laboratory or in a mammal in which the cell resides, a system wherein cells comprising the introduced gene can be used as research, diagnostic and therapeutic tools, and a system wherein animal models are generated which are useful for the development of new diagnostic and therapeutic tools for selected disease states in a mammal.

Such cell expressing an isolated nucleic acid encoding human UPI-resistant UPase can be used to provide human UPI-resistant UPase to a cell, tissue, or whole animal where expression of human UPI-resistant UPase can be useful to treat or alleviate a disease, disorder or condition associated with low level of human UPI-resistant UPase expression and/or activity. Therefore, the invention includes a cell expressing human UPI-resistant UPase to increase or induce human UPI-resistant UPase expression, translation, and/or activity, where increasing human UPI-resistant UPase expression, protein level, and/or activity can be useful to treat or alleviate a disease, disorder or condition.

One of ordinary skill would appreciate, based upon the disclosure provided

herein, that a "knock-in" or "knock-out" vector of the invention comprises at least two sequences homologous to two portions of the nucleic acid which is to be replaced or deleted, respectively. The two sequences are homologous with sequences that flank the gene; that is, one sequence is homologous with a region at or near the 5' portion of the coding sequence of the nucleic acid encoding human UPI-resistant *UPase* and the other sequence is further downstream from the first. One skilled in the art would appreciate, based upon the disclosure provided herein, that the present invention is not limited to any specific flanking nucleic acid sequences. Instead, the targeting vector may comprise two sequences which remove some or all (*i.e.*, a "knock-out" vector) or which insert (*i.e.*, a "knock-in" vector) a nucleic acid encoding human UPI-resistant UPase, or a fragment thereof, from or into a mammalian genome, respectively. The crucial feature of the targeting vector is that it comprise sufficient portions of two sequences located towards opposite, *i.e.*, 5' and 3', ends of the human UPI-resistant UPase open reading frame (ORF) in the case of a "knock-out" vector, to allow deletion/insertion by homologous recombination to occur such that all or a portion of the nucleic acid encoding human UPI-resistant UPase is deleted from or inserted into a location on a mammalian chromosome.

The design of transgenes and knock-in and knock-out targeting vectors is well-known in the art and is described in standard treatises such as Sambrook et al. (1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York), and in Ausubel et al. (1997, Current Protocols in Molecular Biology, John Wiley & Sons, New York), and the like. The upstream and downstream portions flanking or within the human UPI-resistant UPase coding region to be used in the targeting vector may be easily selected based upon known methods and following the teachings disclosed herein based on the disclosure provided herein, including the nucleic and amino acid sequence of human UPI-resistant UPase and the identification and characterization of mutations associated with the UPI-resistant phenotype. Armed with the disclosure provided herein, one of ordinary skill in the art would be able to construct the transgenes and knock-out vectors of the invention.

The invention further includes a knock-out targeting vector comprising a

nucleic acid encoding a selectable marker such as, for example, a nucleic acid encoding the *neo*^{R} gene thereby allowing the selection of transgenic a cell where the nucleic acid encoding human UPI-resistant UPase, or a portion thereof, has been deleted and replaced with the neomycin resistance gene by the cell's ability to grow in the presence of G418. However, the present invention should not be construed to be limited to neomycin resistance as a selectable marker. Rather, other selectable markers well-known in the art may be used in the knock-out targeting vector to allow selection of recombinant cells where the human UPI-resistant UPase gene has been deleted and/or inactivated and replaced by the nucleic acid encoding the selectable marker of choice. Methods of selecting and incorporating a selectable marker into a vector are well-known in the art and are describe in, for example, Sambrook et al. (1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York), and in Ausubel et al. (1997, Current Protocols in Molecular Biology, John Wiley & Sons, New York).

As noted herein, the invention includes a non-human transgenic mammal comprising an exogenous nucleic acid inserted into a desired site in the genome thereof thereby deleting the coding region of a desired endogenous target gene, *i.e.*, a knock-out transgenic mammal. Further, the invention includes a transgenic non-human mammal wherein an exogenous nucleic acid encoding human UPI-resistant UPase is inserted into a site the genome, *i.e.*, a "knock-in" transgenic mammal. The knock-in transgene inserted may comprise various nucleic acids encoding, for example, a tag polypeptide, a promoter/regulatory region operably linked to the nucleic acid encoding human UPI-resistant UPase not normally present in the cell or not typically operably linked to human UPI-resistant UPase.

The generation of the non-human transgenic mammal of the invention is preferably accomplished using the method which is now described. However, the invention should in no way be construed as being limited solely to the use of this method, in that, other methods can be used to generate the desired knock-out mammal.

In the preferred method of generating a non-human transgenic mammal, ES cells are generated comprising the transgene of the invention and the cells are then

used to generate the knock-out animal essentially as described in Nagy and Rossant (1993, In: Gene Targeting, A Practical Approach, pp.146-179, Joyner ed., IRL Press). ES cells behave as normal embryonic cells if they are returned to the embryonic environment by injection into a host blastocyst or aggregate with blastomere stage embryos. When so returned, the cells have the full potential to develop along all lineages of the embryo. Thus, it is possible, to obtain ES cells, introduce a desired DNA therein, and then return the cell to the embryonic environment for development into mature mammalian cells, wherein the desired DNA may be expressed.

Precise protocols for the generation of transgenic mice are disclosed in Nagy and Rossant (1993, In: Gene Targeting, A Practical Approach, Joyner ed. IRL Press, pp. 146-179), and are therefore not repeated herein. Transfection or transduction of ES cells in order to introduce the desired DNA therein is accomplished using standard protocols, such as those described, for example, in Sambrook et al. (1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York), and in Ausubel et al. (1997, Current Protocols in Molecular Biology, John Wiley & Sons, New York). Preferably, the desired DNA contained within the transgene of the invention is electroporated into ES cells, and the cells are propagated as described in Soriano et al. (1991, Cell 64:693-702).

Introduction of an isolated nucleic acid into the fertilized egg of the mammal is accomplished by any number of standard techniques in transgenic technology (Hogan et al., 1986, Manipulating the Mouse Embryo: A Laboratory Manual, Cold Spring Harbor, NY). Most commonly, the nucleic acid is introduced into the embryo by way of microinjection.

Once the nucleic acid is introduced into the egg, the egg is incubated for a short period of time and is then transferred into a pseudopregnant mammal of the same species from which the egg was obtained as described, for example, in Hogan et al. (1986, Manipulating the Mouse Embryo: A Laboratory Manual, Cold Spring Harbor, NY). Typically, many eggs are injected per experiment, and approximately two-thirds of the eggs survive the procedure. About twenty viable eggs are then transferred into

pseudopregnant animals, and usually four to ten of the viable eggs so transferred will develop into live pups.

Any nucleic acid encoding a human UPI-resistant UPase can be used in the methods described herein to produce a transgenic mammal or a transgenic cell harboring a transgene comprising a mutation associated with UPI resistance. Preferably, a nucleic acid encoding human UPI-resistant UPase is used wherein the nucleic acid comprises a mutation associated with UPI-resistance selected from: a change from a C to T at nucleotide 40, a C to T at nucleotide 135, a C to G at nucleotide 178, an A to C at nucleotide 182, a C to T at nucleotide 186, a G to A at nucleotide 713, and a G to A at nucleotide 752.

The transgenic mammal of the invention can be any species of mammal. Thus, the invention should be construed to include generation of transgenic mammals encoding the chimeric nucleic acid, which mammals include mice, hamsters, rats, rabbits, pigs, sheep and cattle. The methods described herein for generation of transgenic mice can be analogously applied using any mammalian species. Preferably, the transgenic mammal of the invention is a rodent and even more preferably, the transgenic mammal of the invention is a mouse. By way of example, Lukkarinen et al. (1997, Stroke 28:639-645), teaches that gene constructs which enable the generation of transgenic mice also enable the generation of other transgenic rodents, including rats. Similarly, nullizygous mutations in a genetic locus of an animal of one species can be replicated in an animal of another species having a genetic locus highly homologous to the first species.

To identify the transgenic mammals of the invention, pups are examined for the presence of the isolated nucleic acid using standard technology such as Southern blot hybridization, PCR, and/or RT-PCR using, among other things, the primers and probes disclosed herein, *e.g.*, nucleic acids having sequences SEQ ID NOs:3-8. Expression of the nucleic acid in the cells and in the tissues of the mammal is also assessed using ordinary technology described herein. Further, the presence or absence of UPI-resistant UPase activity in the tissues, *e.g.*, in the liver tissue, of the transgenic animal can be determined, for example, as disclosed herein (*e.g.*, Western blot analysis),

and using assays to determine UPI-resistant enzymatic activity (*see, e.g.*, Liu et al., 1998, Cancer Res. 58:5418-5424), or using standard methods for protein detection that are well-known in the art.

Cells obtained from the transgenic mammal of the invention, which are also considered "transgenic cells" as the term is used herein, encompass such as cells as those obtained from the UPI-resistant UPase (+/-) and (-/-) transgenic non-human mammal described elsewhere herein, are useful systems for modeling diseases and symptoms of mammals which are believed to be associated with expression of UPI-resistant UPase expression such as breast cancer, head-neck cancer, ovarian cancer, and any other disease, disorder or condition associated with expression of UPI-resistant UPase. Moreover, as a marker of a pathway(s) associated with tumors, *e.g.*, breast, head-neck and ovarian tumors, UPI-resistant UPase expression is also a useful indicator in assessment of such diseases, disorders or conditions.

Particularly suitable are cells derived from a tissue of the non-human knock-out or knock-in transgenic mammal described herein, wherein the transgene comprising the UPI-resistant UPase gene is expressed or inhibits expression of UPI-resistant UPase in various tissues. By way of example, cell types from which such cells are derived include fibroblasts, neurons, hepatocytes, endothelial, adipocyte, and myoblast cells, and the like, of (1) the *UPI-resistant UPase* (+/+), (+/-) and (-/-) non-human transgenic liveborn mammal, (2) the *UPI-resistant UPase* (+/+), (-/-) or (+/-) fetal animal, and (3) placental cell lines obtained from the *UPI-resistant UPase* (+/+), (-/-) and (+/-) fetus and liveborn mammal.

One skilled in the art would appreciate, based upon this disclosure, that cells comprising decreased levels of UPI-resistant UPase protein, decreased levels of UPI-resistant UPase mRNA, decreased level of UPI-resistant UPase activity, or all three, include, but are not limited to, cells expressing inhibitors of UPI-resistant UPase expression (*e.g.*, antisense or ribozyme molecules).

Methods and compositions useful for maintaining mammalian cells in culture are well known in the art, wherein the mammalian cells are obtained from a

mammal including, but not limited to, cells obtained from a mouse such as the transgenic mouse described herein.

The recombinant cell of the invention can be used to study the effect of qualitative and quantitative alterations in UPI-resistant UPase on cell processes. This is because, as disclosed elsewhere herein, UPI-resistant UPase is correlated with certain tumors, *e.g.*, breast, head-neck and ovarian, indicating that UPI-resistant UPase may play a role in cell differentiation and/or growth. Further, the recombinant cell can be used to produce UPI-resistant UPase for use for therapeutic and/or diagnostic purposes. That is, a recombinant cell expressing UPI-resistant UPase can be used to produce large amounts of purified and isolated UPI-resistant UPase for therapeutic and diagnostic uses, and the like.

Alternatively, recombinant cells expressing UPI-resistant UPase can be administered in *ex vivo* and *in vivo* therapies where administering the recombinant cells thereby administers the UPI-resistant UPase protein to a cell, a tissue, and/or an animal. Additionally, the recombinant cells are useful for the discovery of mechanisms associated with UPI-resistant UPase including cellular components that interact and/or are associated therewith.

The recombinant cell of the invention may be used to study the effects of elevated or decreased UPI-resistant UPase levels on cell homeostasis and cell proliferation since the data disclosed elsewhere herein suggests that UPI-resistant UPase may play a role in breast cancer, head-neck cancer, and ovarian cancer, and the like

The recombinant cell of the invention, wherein the cell has been engineered such that it does not express UPI-resistant UPase, or expresses reduced or altered UPI-resistant UPase lacking biological activity, can also be used in *ex vivo* and *in vivo* cell therapies where either an animal's own cells (*e.g.*, hepatocytes, fibroblasts, neurons, and the like) or those of a syngeneic matched donor are recombinantly engineered as described elsewhere herein (*e.g.*, by insertion of an antisense nucleic acid or a knock-out vector such that UPI-resistant UPase expression and/or protein levels are thereby reduced in the recombinant cell), and the recombinant cell is administered to the recipient animal. In this way, recombinant cells that express UPI-resistant UPase at a

reduced level can be administered to an animal whose own cells express increased levels of UPI-resistant UPase thereby treating or alleviating a disease, disorder or condition associated with or mediated by increased UPI-resistant UPase expression, such as, but not limited to, certain tumors (*e.g.*, breast, head-neck and ovarian tumors). Further, the skilled artisan would understand, based upon the disclosure provided herein, that the present invention includes tumorgenesis generally since UPase resistance is not limited solely to the final stage of tumor development. Thus, diseases, disorders and conditions encompass the development of various tumors in breast, head-neck and ovarian, tissues.

One skilled in the art would appreciate, based upon the disclosure provided herein, that recombinant cells and transgenic non-human mammals expressing UPI-resistant UPase provide a model system for the identification of compounds that selectively inhibit wild type UPase but do not inhibit mutant UPI-resistant UPase. Such compounds are important potential therapeutics for use in conjunction with chemotherapeutic agents, *e.g.*, AZT and BAU, that decrease the intracellular pool of uridine since the compounds would protect normal tissues expressing wild type UPase but would not protect tissues expressing mutant UPase from the toxic effects of the chemotherapeutic agent.

VII. Methods

A. Detection of UPI-resistant UPase

The instant invention includes novel assays to detect the presence of human UPI-resistant UPase in a sample and to quantitate the amount of nucleic acid (*i.e.*, RNA, DNA, or both) encoding UPI-resistant *UPase* present in a sample. That is, the invention includes nucleic acid amplification methods such as, but not limited to, PCR-based quantitative sequence detection (QSD), which methods can be used to assess the level of nucleic acid encoding UPase in a sample.

For example, the invention encompasses a PCR-based assay using cDNA, genomic DNA, or both, where the absence or presence of a nucleic acid encoding UPI-

resistant UPase in a sample can be determined.

One skilled in the art would appreciate, based upon the disclosure provided herein, that the nucleic acid sequence should be sufficiently unique to human UPI-resistant *UPase* such that the identical sequence, or a sequence more than 70% homologous to it, does not occur in the sample being tested. By using a sufficiently unique nucleic acid target sequence, any nucleic acid amplification product produced during the amplification phase will not complementary to and, therefore, will not cross-hybridize and/or amplify non-*UPase* nucleic acids under high stringency conditions such as conditions well-known in the art and set forth in, for example, Sambrook et al. (1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York), Ausubel et al. (1997, Current Protocols in Molecular Biology, Green & Wiley, New York).

PCR is the preferred amplification technique used in the amplification step of the present methods. However, the amplification step may also be carried out using any suitable amplification technique known in the art or to be developed. Suitable amplification techniques are described in the following patents, each of which is incorporated herein by reference as if set forth in its entirety: U.S. Pat. Nos. 4,683,195; 4,683,202; 4,965,188; 5,409,818; 5,437,990; 4,957,858; and PCT Patent Publication No. 89/06995.

As described in U.S. Pat. No. 5,770,368, and in standard treatises such as, for example, Sambrook et al., *supra*, and Ausubel et al., *supra*, the preferred PCR amplification procedure used in the present method comprises a target nucleic acid portion unique to a nucleic acid encloding UPI-resistant UPase is amplified by treating the double-stranded target polynucleotide with two oligonucleotide primers, each being complementary to one of the two strands of the target. The primers hybridize with their complementary strands and extension products are synthesized using DNA polymerase and at least four deoxyribonucleotide triphosphates (dNTPs). The extension products are separated from their complementary strands by denaturation at an elevated temperature, typically ranging from about 80°C to about 100°C. The reaction mixture is repeatedly

cycled between a low temperature annealing step usually ranging from about 37°C to about 70°C during which the primers hybridize to their complementary strands, an intermediate temperature (from about 70°C to about 80°C) primer extension step, to the higher temperature denaturation step at a temperature from about 80°C to about 100°C. These temperature steps, or thermal cycling, are repeated many times, typically about 20 to about 40 cycles are carried out, followed by a final synthesis step at about 70°C and a 4°C soak to stop the reaction.

PCR reagents are the chemicals, apart from the target nucleic acid sequence, needed to perform the PCR process. As disclosed by Mayrand (1997, U.S. Pat. No. 5,691,146, these chemicals generally consist of five classes of components: (i) an aqueous buffer, (ii) a water soluble magnesium salt, (iii) at least four deoxyribonucleotide triphosphates (dNTPs) (conventionally, dATP, dTTP, dGTP, dCTP), (iv) oligonucleotide primers (typically two primers for each target sequence, the sequences defining the 5' ends of the two complementary strands of the double-stranded target sequence), and (v) a polynucleotide polymerase, preferably a DNA polymerase, more preferably a thermostable DNA polymerase, *i.e.*, a DNA polymerase which can tolerate temperatures between 90°C and 100°C for a total time of at least 10 minutes without losing more than about half its activity.

Primers for the amplification steps are the same if used for a reverse transcription step at the outset to convert RNA into DNA before carrying out the amplification procedure. Preferably, primers are chosen which only amplify target nucleic acid sequences from a nucleic acid encoding UPI-resistant *UPase*. In the present invention, the primers amplify only a target nucleic acid sequence within the coding region of UPI-resistant *UPase* because they have less sequence similarity to other sequences present in a sample based on the stringency conditions disclosed herein. Further, by selecting forward and reverse primers flanking the region comprising a mutation, the PCR analysis can detect the presence of a mutation.

Preferred primer pairs and probes target the amino-terminal region of the *UPase* coding sequence and include the following: a primer having sequence SEQ ID

NOs: 3-8.

The skilled artisan would appreciate, based upon the disclosure provided herein, that the sequence of the internal oligonucleotide probe used to detect UPI-resistant UPase is not limited to any specific sequences exemplified herein. Thus, once armed with the sequences of UPI-resistant UPase disclosed herein, one skilled in the art would be able to design appropriate primer pairs and internal probe sequences following methods well-known in the art and taught elsewhere herein.

It will be understood by those skilled in the art based on this disclosure that in the present invention, the target nucleic acid and the portion of the amplified target sequence to which the PCR oligonucleotide probe hybridizes are unique to UPI-resistant *UPase*, or both, such that the probe and primers do not hybridize to non-UPase nucleic acids under conditions of high stringency. Thus, the nucleic acid-based detection method of the present invention only detects amplification of the specific, unique *UPase* target nucleic acid sequence and not that of other sequences which may be present in the sample.

In the standard PCR assay, the amplified target nucleic acid sequence can be detected directly by any method that can distinguish among the different lengths of DNA. Electrophoresis through agarose gels is the standard method known in the art for use in separating, identifying, and purifying DNA fragments following PCR. The location of the DNA within the gel can be determined directly by staining the gel with low concentrations of the intercalating fluorescent dye, ethidium bromide (EtBr). Band(s) corresponding to the predicted length for the amplified target DNA can then be detected by direct examination of the gel in ultraviolet light.

Additionally, the DNA bands from an electrophoresed sample can be probed by Southern blotting using a single-stranded oligonucleotide probe which is complementary to a sequence located between the two selected oligonucleotide primers in the amplified target nucleic acid sequence. Usually, the oligonucleotide probe is labeled with a radioactive or fluorescent tag, or attached directly or indirectly to an enzyme molecule such that the probe specifically bound to the immobilized complementary target

sequence may be localized.

In the preferred embodiment herein, the oligonucleotide probe was complementary to a specific portion of the UPI-resistant *UPase* coding sequence. However, the present invention is not limited to this sequence. Rather, the oligonucleotide probe can be selected to hybridize to any target amplified nucleic acid located between two primer pairs all of which hybridize to a sequence in UPI-resistant *UPase* but which do not hybridize to a nucleic acid that does not encode UPI-resistant *UPase* that can be present in a sample of interest.

The oligonucleotides used in the invention can be synthesized using any standard method known or to be developed. Suitable syntheses are described in Ozaki et al. (1992, Nucleic Acids Res. 20:5205-5214) and Agrawal et al. (1990, Nucleic Acids Res. 18:5419-5423).

The oligonucleotide probes of the invention are preferably conveniently synthesized on an automated DNA synthesizer such as a Perkin-Elmer (Foster City, CA) Model 392 or 394 DNA/RNA synthesizer using standard chemistries, such as phosphoramidite chemistry described in Beaucage and Iyer (1992, Tetrahedron 48:2223-2311), Molko et al. (U.S. Pat. No. 4,980,460), Koster et al. (U.S. Pat. No. 4,725,677), Caruthers et al. (U.S. Pat. Nos. 4,415,732 and 4,458,066). However, other similar syntheses using chemistries and techniques may be used. Alternative chemistries resulting in non-natural backbone groups, such as phosphorothioate, phosphoramidate, and the like, may also be used provided the hybridization efficiencies of the resulting oligonucleotides are not adversely affected.

Preferably, the oligonucleotide probe is in the range of about 15 to about 150 nucleotides in length. The precise sequence and length of an oligonucleotide probe of the invention depends in part on the nature of the target nucleic acid sequence to which it hybridizes. The binding location and length may be varied to achieve appropriate annealing and melting properties for a particular embodiment by one skilled in the art in accordance with known techniques such as "TaqMan-type" assays.

Oligonucleotides of the present invention include linear oligomers of

natural or modified monomers or linkages, such as deoxyribonucleotides, ribonucleotides, and the like, which are capable of specifically binding to a target polynucleotide by way of a regular pattern of monomer-to-monomer interactions, such as Watson-Crick base pairing. Usually, monomers are linked by phosphodiester bonds or their analogs to form oligonucleotides ranging in size from a few monomeric units, *e.g.*, 3-4, to several tens of monomeric units. Whenever an oligonucleotide is represented by a sequence of letters, such as "ATGCCTG," it will be understood that the nucleotides are in a 5'→3' order from left to right and that "A" denotes deoxyadenosine, "C" denotes deoxycytidine, "G" denotes deoxyguanosine, and "T" denotes thymidine, unless otherwise noted. Analogs of phosphodiester linkages include phosphorothioate, phosphoranilidate, phosphoramidate, and similar compounds.

The present invention further includes detection of UPI-resistant UPase using methods to assess the level of UPase biological activity in the absence or presence of various levels of at least one UPI. Thus, detection of presence or absence of UPI-resistant UPase in a sample is not limited to using nucleic acid-based methods. Rather, one skilled in the art would appreciate, based upon the disclosure provided herein, that the present invention is not limited to any particular method or assay for detecting UPI-resistant UPase. That is, the present invention includes detecting a nucleic acid encoding UPI-resistant UPase as well as detecting UPI-resistant UPase enzymatic activity by any means available in the art.

Methods for detecting a protein of interest such as UPI-resistant UPase are also well known in the art and are encompassed herein to assess presence or absence of UPI-resistant UPase in a biological sample.

In one aspect, the invention encompasses detecting a UPI-resistant UPase using an antibody or other molecular or chemical binding partner that specifically binds therewith. The presence of UPI-resistant UPase can be detected and/or quantitated using a number of well-defined diagnostic assays. Those skilled in the art can adapt any of the conventional immunoassay formats to detect and/or quantitate the amount of a UPI-resistant UPase protein, such as, for example, a competitive radioimmunoassay. Many

other formats for detection or quantitation of a UPI-resistant UPase protein using UPI-resistant UPase-specific antibodies are, of course available. These include, but are not limited to, Western blotting, ELISA (sandwich enzyme-linked immunosorbent assay), RIA (competitive radioimmunoassay), EIA (competitive enzyme immunoassay), IRMA (sandwich immunoradiometric assay), dual antibody sandwich assays, and other assays all commonly used in the diagnostic industry and any means used to produce signal including enzymes, fluorophores, metals, chemi-, bio-, or electroluminescent compounds and any signal amplification systems such as biotin-(strept)avidin, and the like. In such immunoassays, the interpretation of the results is based on the assumption that the antibody or antibody combination will not cross-react with other proteins and protein fragments present in the sample that are unrelated to UPI-resistant UPase.

Antibodies employed in assays may be labeled or unlabeled. Unlabeled antibodies may be employed in agglutination; labeled antibodies may be employed in a wide variety of assays, employing a wide variety of labels. Suitable detection means include the use of labels such as radionuclides, enzymes, coenzymes, fluorescers, chemiluminescers, chromogens, enzyme substrates or co-factors, enzyme inhibitors, free radicals, particles, dyes and the like. Such labeled reagents may be used in a variety of well known assays, such as radioimmunoassays, enzyme immunoassays (*e.g.*, ELISA), fluorescent immunoassays, and the like. *See, e.g.*, U.S. Pat. Nos. 3,766,162; 3,791,932; 3,817,837; and 4,233,402.

Additionally, numerous competitive and non-competitive protein binding assays have been described in the scientific and patent literature, and a large number of such assays are commercially available. Exemplary immunoassays which are suitable for detecting a UPI-resistant UPase include those described in U.S. Pat. Nos. 3,791,932; 3,817,837; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; and 4,098,876.

Thus, the present invention encompasses methods of detecting UPI-resistant UPase, or a component or portion thereof, using an antibody, chemical, and other molecule that specifically binds with UPI-resistant UPase. Further, the invention

encompasses detection of UPI-resistant UPase using nucleic acid methods such as those disclosed elsewhere herein, known in the art, and such methods as are developed in the future.

Moreover, the present invention encompasses detection of UPI-resistant UPase using detection methods based on assessing the presence or absence of UPase enzymatic activity, in the presence or absence of a UPI.

The invention further includes a method of identifying a human afflicted with a disease, disorder or condition associated with expression of UPI-resistant UPase. The method comprises detecting a mutation in a nucleic acid encoding UPase which mutation is associated with resistance to a uridine phosphorylase inhibitor. This is because, as disclosed elsewhere herein, applicants have discovered that the UPase in certain tumors is resistant to inhibition by UPIs. Therefore, the correlation between UPI resistance and the presence of a tumor can be used to detect such tumor by detecting the presence of the UPI-resistant UPase. As discussed previously elsewhere herein, the presence of UPI-resistant UPase can be determined using a variety of assay methods including methods based on detection of nucleic acids comprising mutations that are associated with UPI-resistance, as well as methods based on detection of UPI-resistant UPase enzymatic activity, among others.

Therefore, because UPI resistance in UPase obtained in a tissue is correlated with the presence of a tumor in the tissue, by detecting the presence of UPI-resistant UPase in a human, a human suffering from a disease, disorder, or condition associated with expression of UPI-resistant UPase (*e.g.*, breast, head-neck and ovarian tumors) can be detected and diagnosed.

B. Methods of detecting mutations in the *UPase* gene locus

The methods of the invention can be used to detect mutations in a nucleic acid of the invention in order to determine if a human has a mutated gene since such a mutation is correlated with a disease, disorder, or condition associated with UPI-resistant UPase, *e.g.*, tumors in certain tissues such as breast, head-neck, and ovarian

tumors. This is because the present invention teaches, for the first time, that the presence of UPI-resistant UPase is correlated with certain diseases, disorders and conditions, and because it has been further discovered, as disclosed elsewhere herein, that certain mutations in the coding region of UPase are associated with resistance to UPI. Therefore, the skilled artisan would appreciate, based upon the disclosure provided herein, that by detecting a mutation associated with UPI-resistance, and thereby detecting a nucleic acid encoding a UPI-resistant UPase, a disease, disorder or condition can be detected. Such diseases, disorders and conditions include, *e.g.*, breast tumors, head-neck tumors, and ovarian tumors.

In certain embodiments, the methods include detecting, in a biological sample obtained from the human, the presence or absence of a mutation characterized by at least one of an alteration of a nucleic acid encoding a human UPase of the invention, or the expression of a UPase that is resistant to a UPI compared with wild type UPase.

Such mutations can be detected by ascertaining the existence of at least one of: 1) a deletion of one or more nucleotides from the nucleic acid encoding *UPase*; 2) an addition of one or more nucleotides to the nucleic acid encoding *UPase*, 3) a substitution of one or more nucleotides of the nucleic acid encoding *UPase*; 4) a chromosomal rearrangement of the nucleic acid encoding *UPase*; 5) an alteration in the level of a messenger RNA transcript of the nucleic acid encoding *UPase*; 6) an aberrant modification of the gene, such as of the methylation pattern of the genomic DNA; 7) a non-wild type splicing pattern of a messenger RNA transcript of the nucleic acid encoding *UPase*; 8) a non-wild type level of the protein encoded by the nucleic acid encoding *UPase*; 9) an allelic loss of the nucleic acid encoding *UPase*; and 10) an inappropriate post-translational modification of the protein encoded by the nucleic acid encoding *UPase*. As described herein, there are a large number of assay techniques known in the art which can be used for detecting such mutations in a nucleic acid encoding a known protein. Thus, once armed with the teachings set forth herein, including the nucleic and amino acid sequences of UPI-resistant UPase and wild type

UPase, and the full length sequence of human *UPase* gDNA disclosing the genomic arrangement of the entire sequence, one skilled in the art would be able to detect a mutation in the *UPase* gene.

In certain embodiments, detection of the mutation involves the use of an primer in a polymerase chain reaction (PCR; *see, e.g.*, U.S. Patent Nos. 4,683,195 and 4,683,202), such as anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR; *see, e.g.*, Landegran et al., 1988, Science 241:1077-1080; and Nakazawa et al., 1994, Proc. Natl. Acad. Sci. USA 91:360-364), the latter of which can be particularly useful for detecting point mutations in a gene (*see, e.g.*, Abravaya et al., 1995, Nucleic Acids Res. 23:675-682). This method can include the steps of collecting a sample of cells from a patient, isolating nucleic acid (*e.g.*, genomic, mRNA, or both) from the cells of the sample, contacting the nucleic acid sample with one or more primers which specifically hybridize with the selected gene under conditions such that hybridization and amplification of the gene (if present) occurs, and detecting the presence or absence of an amplification product. The method can also include detecting the size of the amplification product and comparing the length to the length of a corresponding product obtained in the same manner from a control sample. PCR, LCR, or both can be used as a preliminary amplification step in conjunction with any of the techniques used for detecting mutations described herein.

Alternative amplification methods include: self-sustained sequence replication (Guatelli et al., 1990, Proc. Natl. Acad. Sci. USA 87:1874-1878), transcriptional amplification system (Kwoh, et al., 1989, Proc. Natl. Acad. Sci. USA 86:1173-1177), Q-Beta Replicase (Lizardi et al., 1988, Bio/Technology 6:1197), or any other nucleic acid amplification method, followed by the detection of the amplified molecules using any of a variety of techniques well known to those of skill in the art. These detection schemes are especially useful for detection of nucleic acid molecules if such molecules are present in very low numbers.

In an alternative embodiment, mutations in a selected gene can be identified in a sample by detecting alterations in restriction enzyme cleavage patterns.

For example, sample and control DNA is isolated, (optionally) amplified, digested with one or more restriction endonucleases, and fragment length sizes are determined by gel electrophoresis and compared. Differences in fragment length sizes between sample and control DNA (*i.e.*, restriction fragment length polymorphism, RFLP) indicates occurrence of mutations or other sequence differences in the sample DNA compared with control, wild type DNA.

Moreover, sequence specific ribozymes (*see, e.g.*, U.S. Patent No. 5,498,531) can be used to detect the presence of specific mutations by development or loss of a ribozyme cleavage site.

In other embodiments, genetic mutations are identified by hybridizing a sample and control nucleic acids, *e.g.*, DNA or RNA, with high density arrays containing hundreds or thousands of oligonucleotides probes (Cronin et al. (1996) Human Mutation 7:244-255; Kozal et al. (1996) Nature Medicine 2:753-759.

In addition, any of a variety of sequencing methods known in the art can be used to directly sequence the selected gene and detect mutations by comparing the sequence of the sample nucleic acids with the corresponding wild-type (control) sequence (*see, e.g.*, Maxam and Gilbert, 1977, Proc. Natl. Acad. Sci. USA 74:560; Sanger, 1977, Proc. Natl. Acad. Sci. USA 74:5463. It is also contemplated that any of a variety of automated sequencing procedures can be used when performing the diagnostic assays (as reviewed in 1995, Bio/Techniques 19:448). Such automated sequencing methods include mass spectrometry (*see, e.g.*, PCT Publication No. WO 94/16101; Cohen et al., 1996, Adv. Chromatogr. 36:127-162; Griffin et al., 1993, Appl. Biochem. Biotechnol. 38:147-159).

Other methods for detecting mutations in a selected gene include methods involving protection from cleavage agents to detect mismatched bases in RNA / RNA or RNA / DNA heteroduplexes as described in, *e.g.*, Myers et al. (1985, Science 230:1242). In essence, hybridizing RNA or DNA containing wild-type sequence with potentially mutant RNA or DNA obtained from a tissue sample and subsequent treatment of the duplexes formed with an agent(s) (*e.g.*, S1 nuclease, hydroxylamine or

osmium tetroxide with piperidine, DNA mismatch enzymes such as mutY from *E. coli* or mammalian thymidine DNA glycosylase) that cleaves single-stranded regions of duplex detects base pair mismatches between the control and sample strands. Following digestion of the mismatched regions, the resulting material is separated by size on denaturing polyacrylamide gels to determine the site of the mutated or mismatched region (*see, e.g.*, Cotton et al., 1988, Proc. Natl. Acad. Sci. USA 85:4397; Saleeba et al., 1992, Methods Enzymol. 217:286-295).

In other embodiments, alterations in electrophoretic mobility are used to identify mutations in genes. For example, single strand conformation polymorphism (SSCP) analysis can be used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids as described in Orita et al. (1989, Proc. Natl. Acad. Sci. USA 86:2766), Cotton (993, Mutat. Res. 285:125-144), and Hayashi (1992, Genet. Anal. Tech. Appl. 9:73-79).

Techniques such as the use of Cleavase as disclosed elsewhere herein, can be used to detect a mutation in the UPase gene.

In yet another embodiment, the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel electrophoresis (DGGE), as described (Myers et al., 1985, Nature 313:495).

Examples of other techniques for detecting point mutations include, but are not limited to, selective oligonucleotide hybridization, selective amplification, and selective primer extension (*see, e.g.*, Saiki et al., 1986, Nature 324:163; Saiki et al., 1989, Proc. Natl. Acad. Sci. USA 86:6230).

Alternatively, allele specific amplification technology can be used in conjunction with the methods of the invention as described in, for example, Gibbs et al. (1989, Nucleic Acids Res. 17:2437-2448), Prossner (1993, Tibtech 11:238), Gasparini et al. (1992, Mol. Cell Probes 6:1), and Barany (1991, Proc. Natl. Acad. Sci. USA 88:189).

Additional methods of detecting a mutation include fluorescent *in situ*

hybridization (FISH), as disclosed elsewhere herein, and similar methods well-known in the art.

The methods described herein can be performed, for example, using pre-packaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent described herein. Such kits can be used, for example, to diagnose a human patient exhibiting a disease, disorder, or condition involving a nucleic acid encoding UPI-resistant UPase. Furthermore, any cell type or tissue in which the polypeptide of the invention is expressed, *e.g.*, a breast tumor sample, a head-neck tumor sample, an ovarian tumor sample, and the like, can be used in the prognostic assays described herein.

C. Methods of diagnosis of cancer, monitoring for recurrence, monitoring for effectiveness of therapy, and assessment of disease stage, grade, and prognosis

The present invention includes methods of diagnosis of certain diseases, disorders, or conditions (*e.g.*, breast, head-neck and ovarian tumors, among others) which are associated with or mediated by an increased resistance of UPase to a UPI.

The invention includes a method of detecting (*i.e.*, diagnosing) a cancer in a previously undiagnosed human. The method comprises obtaining a biological sample from a human and detecting the presence or absence of UPase resistant to UPI. The UPI-resistant UPase can be detected by detecting either the presence of a mutation associated with UPI resistance in a nucleic acid encoding the UPase or by detecting resistance to the inhibitory effects of UPI on UPase enzymatic activity.

This is because, as disclosed elsewhere herein, an increased resistance of UPase to UPI is correlated to certain tumors such that detecting UPI-resistant UPase in a biological sample indicates the presence of a tumor in such sample.

One skilled in the art would understand, based upon the disclosure provided herein, that a standard baseline level, reference range, or normal resistance to UPI can be determined for any given human or tissue. Such a reference level of UPI sensitivity to UPI inhibition can be used in the comparison to identify a human having a

UPase resistant to UPI, *i.e.*, a level that is arbitrarily and/or statistically greater than the standard baseline level of resistance to UPI.

Alternatively, a standard level of UPI sensitivity exhibited by UPase can be determined in parallel with the sample being assessed. That is, the level of UPI-resistant UPase activity in the biological sample at issue can be compared with the level of UPI-resistant UPase activity present in an otherwise identical biological sample obtained and processed contemporaneously with the sample being assessed and obtained from, *e.g.*, tissue adjacent to a tumor.

Similarly, the nucleic acid encoding a UPase obtained from a tumor tissue can be compared to the nucleic acid encoding a UPase obtained from an adjacent normal tissue where a difference in the nucleic acid sequences of the nucleic acids is an indication that the tumor tissue comprises a mutation associated with UPI-resistance.

Achievement of baseline values of UPI-sensitivity derived from a control population of healthy individuals or baseline values derived from normal tissues can be compared to the UPI-resistant UPase activity in a human following anti-cancer treatment and can indicate successful therapy and provide an endpoint to treatment. Accordingly, detection of UPI-resistant UPase (*i.e.*, detection of nucleic acids having a mutation associated with UPI-resistance and/or UPI-resistant UPase activity) can be measured to determine the most effective therapy regimens for a human and aid in reducing cancer recurrence.

A human in remission can be monitored UPI-resistant UPase (nucleic acid mutation and/or UPI-resistant UPase activity in a biological sample) during follow-up visits. An increase in UPI-resistant UPase can detect a relapse and earlier detection can expedite earlier treatment, which is more effective.

The invention further includes a method of assessing the effectiveness of a treatment for a tumor in a human. The method comprises assessing the level of UPI-resistant UPase (by detecting a mutation associated with such resistance or by assessing the resistance to UPI of the enzymatic activity of UPase obtained from a sample from the human), before, during and after a specified course of treatment for a disease, disorder or

condition mediated by or associated with increased UPase resistance to UPI (*e.g.*, breast, head-neck and ovarian cancer, and the like). This is because, as stated previously elsewhere herein, UPase resistance to UPI is associated with the presence of a tumor. Thus, assessing the effect of a course of treatment based upon UPI-resistant UPase (nucleic acids comprising a mutation associated with UPI-resistance or detecting UPI-resistant UPase enzymatic activity) indicates the efficacy of the treatment such that a lower level of UPI-resistant UPase in a sample taken from a human during and/or after the administration of anti-cancer therapy to that human indicates that the treatment method is successful and either no change or an increase in UPI-resistant UPase level indicates that the therapy is not successful and should be discontinued or modified.

Thus, in addition to the aforementioned clinical utilities in cancer diagnosis and prognosis, detection of UPI-resistant UPase is useful for monitoring anti-cancer therapy effectiveness.

In sum, the invention provides methods and compositions for evaluating the probability of the presence of malignant cells indirectly by detecting UPI-resistant UPase (*e.g.*, by detecting a nucleic acid mutation associated with UPI-resistant UPase and/or by detecting UPI-resistant UPase activity) in, for example, a biological sample removed from a human. Such an assay can be used to detect tumors, and help in the diagnosis and prognosis of disease. The assays can also be used to detect the presence of cancer metastasis, as well as to confirm the absence or removal of tumor tissue following surgery, cancer chemotherapy and/or radiation therapy. It can further be used to monitor cancer chemotherapy and tumor reappearance.

The assays of this invention can be used for both diagnostic and/or prognostic purposes, *i.e.*, diagnostic/prognostic. The term "diagnostic/prognostic" is used herein to encompass the following processes either individually or cumulatively depending upon the clinical context: determining the presence of disease, determining the extent of the malignant disease (stage), distinguishing malignant disease from another disease, forecasting the probable outcome of a disease state, determining the prospect as to recovery from a disease as indicated by the nature and symptoms of a case, monitoring

the disease status of a human, monitoring a human for recurrence of disease, determining the preferred therapeutic regimen for a human, and/or determining the suitability of a particular human for effective therapy with any specific drug or other treatment, including but not limited to surgery, chemotherapy, and the like, or combination of drugs and/or other treatments, including but not limited to surgery, chemotherapy, and the combination of UPI therapy with a chemotherapeutic regimen, and the like.

The present invention includes a method treating cancer in a human receiving 5-fluorouracil as a chemotherapeutic agent. The method comprises assessing whether the tumor comprises a UPI-insensitive UPase. This is because the presence of UPI-resistant UPase in a tumor indicates that the tumor is more sensitive to the toxic effects of 5-FU than the adjacent tissue which expresses normal UPase which is sensitive to UPI. As disclosed previously elsewhere herein, UPase inhibitors ameliorate the toxic effects of 5-FU by mediating an increase in the level of plasma and intracellular uridine. Where the tumor tissue comprises UPase that is not inhibited by UPIs such as, but not limited to, BAU, the tumor tissue will be more susceptible to the toxic effects of 5-FU than the adjacent normal tissue. Thus, detection of UPI-resistant UPase in a tumor sample indicates that a UPI, *e.g.*, BAU, should be co-administered with 5-FU thereby increasing the efficacy of the treatment and, thereby, proving a method of treating cancer in an patient receiving 5-FU.

One skilled in the art would understand, based upon the disclosure provided herein, that the present invention encompasses a method of treating a cancer relating to determining whether UPI should be co-administered with an anticancer agent in a therapy regimen. That is, the present invention provides methods of assessing whether a tumor is UPI resistant or not. One skilled in the art would appreciate that administration of UPI along with an chemotherapy agent where the tumor is not resistant to UPase can actually rescue the tumor tissue along with the normal tissue thereby decreasing the effect of the chemotherapy agent. Indeed, it is long-recognized in the art that administration of BAU simultaneous with, or shortly before or after, administration of 5-FU actually decreases the antitumor effect of 5-FU. Instead, BAU is most effective

at rescuing normal tissue while permitting 5-FU to affect tumor cells when administered about 2 to 24 hours after the administration of 5-FU. Thus, the present invention provides an important advance in the design of methods effective to treat or alleviate cancer by providing a method whereby the potential benefit and detriment of administering a UPI during anticancer therapy can be readily assessed in order to maximize the effectiveness of the therapy.

The diagnostic/prognostic methods of this invention are useful, for example, for screening populations for the presence of neoplastic disease. One skilled in the art would appreciate, based upon the disclosure provided herein that this test will not predict risk; instead, it can detect a malignancy that already exists, thereby diagnosing the presence of neoplastic disease, monitoring the disease status of a human afflicted with certain tumors, assessing whether a UPI should be administered along with the chemotherapeutic agent 5-FU, and/or determining the prognosis for the course of cancer, and the like.

VIII. Kits

The invention includes various kits which comprise a compound, such as an isolated nucleic acid that detects a mutation associated with UPI-resistant UPase, and instructional materials which describe use of the compound to perform the methods of the invention. Although exemplary kits are described below, the contents of other useful kits will be apparent to the skilled artisan in light of the present disclosure. Each of these kits is included within the invention.

The invention encompasses a kit for detecting a nucleic acid encoding human uridine phosphorylase resistant to a uridine phosphorylase inhibitor. The kit comprises at least one nucleic acid selected from the nucleic acids having the following sequences: SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:8. The kit further includes an instructional material for the use thereof. This kit is useful for detecting a nucleic acid encoding a UPI-resistant UPase since, as

disclosed previously elsewhere herein, these nucleic acids, when used according to the teachings of the present invention, detect a mutation associated with UPI-resistance of UPase.

One skilled in the art would understand, based upon the disclosure provided herein, that the nucleic acids can be used in various combinations and permutations which would be understood by one of ordinary skill in the art armed with the disclosure provided herein.

The invention includes a kit for detecting a cancer in a human. The kit comprises at least one nucleic acid selected from the following group: a nucleic acid having the sequence SEQ ID NO:3, a nucleic acid having the sequence SEQ ID NO:4, a nucleic acid having the sequence SEQ ID NO:5, a nucleic acid having the sequence SEQ ID NO:6, a nucleic acid having the sequence SEQ ID NO:7, and a nucleic acid having the sequence SEQ ID NO:8. The kit further includes an instructional material for the use thereof.

One skilled in the art would understand, based upon the disclosure provided herein, that a cancer can be detected using these nucleic acids since the nucleic acids can detect a mutation in a nucleic acid encoding UPase which mutation is associated with UPI-resistance and further since the present invention discloses, for the first time, that UPI-resistant UPase is associated with certain cancers, *e.g.*, breast, head-neck, and ovarian cancer. Therefore, detecting a mutation that detects a UPI-resistant UPase can be used to detect a cancer where the presence of the cancer in a tissue is correlated to the presence in the tissue of UPI-resistant UPase.

The invention includes a kit for monitoring the treatment of a human previously diagnosed with cancer. The kit comprises at least one nucleic acid selected from the group as follows: a nucleic acid having the sequence SEQ ID NO:3, a nucleic acid having the sequence SEQ ID NO:4, a nucleic acid having the sequence SEQ ID NO:5, a nucleic acid having the sequence SEQ ID NO:6, a nucleic acid having the sequence SEQ ID NO:7, and a nucleic acid having the sequence SEQ ID NO:8. The kit includes an instructional material for the use thereof.

As discussed previously elsewhere herein, the kit is based on the discovery disclosed herein that certain mutations in a nucleic acid are associated with UPI-resistance of UPase and that such UPI-resistance is associated with the presence of a tumor. This discovery is an important breakthrough in the development of anticancer diagnostics and therapeutics as more fully set forth elsewhere herein.

Similarly, the invention includes a kit for identifying a human afflicted with a disease, disorder or condition associated with expression of uridine phosphorylase resistant to a uridine phosphorylase inhibitor. The kit comprising at least one nucleic acid selected from the group consisting of a nucleic acid having the sequence SEQ ID NO:3, a nucleic acid having the sequence SEQ ID NO:4, a nucleic acid having the sequence SEQ ID NO:5, a nucleic acid having the sequence SEQ ID NO:6, a nucleic acid having the sequence SEQ ID NO:7, and a nucleic acid having the sequence SEQ ID NO:8. Also, the kit comprises an instructional material for the use thereof.

Additionally, the invention includes a kit for detecting a mutation in a uridine phosphorylase allele in a human where the kit comprises at least one nucleic acid selected from the group consisting of a nucleic acid having the sequence SEQ ID NO:3, a nucleic acid having the sequence SEQ ID NO:4, a nucleic acid having the sequence SEQ ID NO:5, a nucleic acid having the sequence SEQ ID NO:6, a nucleic acid having the sequence SEQ ID NO:7, and a nucleic acid having the sequence SEQ ID NO:8, and an instructional material for the use thereof.

Detection of such mutations is useful in that as more fully set forth previously herein, certain mutations are associated with the presence of various tumors, such that detecting the mutation detects the tumor.

The invention encompasses a kit for treating cancer in a human receiving 5-fluorouracil. The kit comprises at least one nucleic acid selected from the group consisting of a nucleic acid having the sequence SEQ ID NO:3, a nucleic acid having the sequence SEQ ID NO:4, a nucleic acid having the sequence SEQ ID NO:5, a nucleic acid having the sequence SEQ ID NO:6, a nucleic acid having the sequence SEQ ID NO:7,

and a nucleic acid having the sequence SEQ ID NO:8. The kit further comprises an instructional material for the use thereof.

One skilled in the art would appreciate, based upon the disclosure provided herein, that other nucleic acids can be used in the kits of the invention. That is, the present invention encompasses kits comprising a nucleic acid wherein the nucleic acid comprises at least one a mutation selected from the group consisting of a C to T at nucleotide 40, a C to T at nucleotide 135, a C to G at nucleotide 178, an A to C at nucleotide 182, a C to T at nucleotide 186, a G to A at nucleotide 713, and a G to A at nucleotide 752. The kits include kits comprising any combination of at least one nucleic acid of the invention.

The kit is useful since, as discussed previously elsewhere herein, detection of certain mutations associated with UPI-resistant UPase, detect the presence of certain tumors. Further, detecting UPI-resistant UPase is useful in indicating those tumors that are more susceptible to the toxic effects of 5-FU since the tumor tissue UPase is not inhibited by a UPI while the UPase present in the adjacent normal tissue is inhibited thereby protecting the normal tissue from the toxic effects of 5-FU by maintaining or increasing the intracellular pool of uridine in normal tissue but not in tumor tissue.

Each kit has appended thereto, an instructional material which describes the use of the kit. The instructional material included in a kit of the invention can be a publication, a recording, a diagram, or any other medium of expression which can be used to communicate one or more of the steps of any analytical, diagnostic, or therapeutic method included in the present invention. The instructional material can also include any medium of expression which is used to communicate the instructions for use of the kit which describe the steps of a method of the invention, and troubleshooting indications and precautions as would be obvious to one skilled in the art after reading the present disclosure.

The instructional material of a kit included in the invention may comprise a plurality of materials which can be provided with one or more components of the kit. By way of example, a kit which is useful for diagnosing a tumor or cancer in a human can

include an instructional material which outlines the method of detecting a nucleic acid comprising a mutation associated with UPI-resistant UPase in a biological sample as described previously elsewhere herein. Kits described herein can also include instructions for assessing the efficacy of anti-tumor therapy by assessing the change, if any, in the level of UPI-resistant UPase activity (or the presence of a nucleic acid encoding UPase comprising a mutation associated with UPI resistance) in a biological sample obtained from a human being treated with the therapy where the level of UPI-resistant UPase is assessed before, during, and/or after the therapy is administered to the human as disclosed elsewhere herein.

The invention is further described in detail by reference to the following experimental examples. These examples are provided for purposes of illustration only, and are not intended to be limiting unless otherwise specified. Thus, the invention should in no way be construed as being limited to the following examples, but rather, should be construed to encompass any and all variations which become evident as a result of the teaching provided herein.

EXAMPLES

Example 1: Identification and characterization of BAU-resistant UPase in tumor tissues

The disclosure provided in Liu et al., 1998, Cancer Res. 58:5418-5424, is incorporated by reference as if set forth in its entirety herein.

Briefly, during evaluation of UPase enzymatic activity associated with tumors, it was discovered that UPase activity was variable among different tissue specimens but overall it was 2-3 fold higher in tumors compared to the paired normal tissue (Figure 1) (Liu et al., 1998, Cancer Res. 58:5418-5424). That is, UPase was isolated from normal and tumors as described in Liu et al. (1998, Cancer Res. 58:5418-5424), including purification using a 5'-NH_2-BAU affinity column. More specifically, an affinity matrix was prepared using an NH_2 derivative of BAU (5'-amino-benzylacyclouridine) coupled through *N*-hydroxy-succinimide chemistry to an agarose

matrix (Affigel-10, Bio-Rad Hercules, CA).

Particle free extracts of murine colon tumor 26 were used as an initial source of UPase. Using a 0.2 ml column to which about 5 μmol of amino-BAU were coupled (the remainder of the sites were blocked using ethanolamine), up to 20 ml of the supernatant from a 5:1 homogenate could be stripped of phosphorylase activity. After thorough washing, the column was eluted with 20 mM uridine to yield a single major protein band at M_r of about 32,000 to 35,000, which contained about 20-40% of the original UPase activity. When combined with a preliminary DEAE column purification (adsorption and elution with 0.2 mM NaCl), a homogeneous protein was obtained as detected using SDS-PAGE analysis (*see, e.g.*, Figure 1 of Liu et al., 1998, Cancer Res. 58:5418-5424) and isoelectric focusing data. Therefore, a 7000-fold purification with a greater than 20% yield was achieved using only two steps.

The purified murine UPase was used to produce polyclonal antisera in rabbits. The antibody recognized a single band at about M_r of about 32,000 to 35,000 in Western blot analysis demonstrating that the antisera was specific for UPase (*id.* at Figure 2). Western blot analysis also demonstrated that UPase level was greater in tumor tissue compared with normal otherwise identical adjacent non-tumor tissue (*id.* at Figure 3).

In addition, human UPase cDNA was cloned from a human liver expression library confirming that the nucleic acid sequence of the clone was identical in the open reading frame to that previously described in Watanabe et al. (1995, Biochem. Biophys. Res. Commun. 216:265-272) but with a longer 5' untranslated region.

In order to express and purify human recombinant UPase, the UPase cDNA codon region was inserted into a pMal C2 vector (New England BioLabs, Beverly, MA) at the EcoRII-HindIV cut sites, which encodes for a maltose binding fusion protein. A single $DH_{5\alpha}$ colony transformed with the plasmid containing the UPase gene was grown overnight in rich medium (tryptone, yeast extract, NaCl and glucose) containing 100 μg/ml ampicillin. The overnight culture was diluted 1:100 in rich medium containing ampicillin and grown until A_{600} reached 0.5. Growth was induced using 0.3 mM isopropyl-1-thio-β-D-galactopyranoside (IPTG) for 2 hours at 37°C. Bacteria were

pelleted using centrifugation for 15 minutes at 7,000 x g at 4°C. Pellets were resuspended in column buffer (20 mM Tris-HCl (pH 7.5), 200 mM NaCl, 1 mM EDTA, and 10 mM beta-mercaptoethanol). The cells were lysed using a French press, and the lysate was clarified using centrifugation at 10,000 x g (1 hour at 4°C) using a JA-20 rotor (Sorvall, Newtown, CT). Supernatants were applied to an amylose resin column, which binds the maltose binding protein (MBP) component of the fusion protein. The fusion protein bound to the column was eluted using 10 mM maltose in column buffer and then cleaved by 0.1% factor Xa at 4°C for 20 hours. The fusion protein cleavage mixture was dialyzed with 20 mM Tris-HCl, 25 mM NaCl (pH 7.5) using three changes of 100 mls volumes for 2 hours. The mixture was then applied to a DEAE column. Maltose binding protein and trace contaminants were retained by the DEAE resin, and pure recombinant UPase was collected in the flow-through. The pure protein was then concentrated using Centricon-10 microconcentrators (Amicon, Beverly, MA).

Furthermore, in all normal tissues and most tumor specimens evaluated, the UPase activity was very sensitive to the effect of BAU with approximately 90-95% inhibition in the presence of 10 μM BAU (*see id.*). However, all of the breast tumors specimens examined (n=28), as well as head-neck and ovarian tumor samples assayed demonstrated partial sensitivity to the inhibitor with approximately 40% of residual phosphorolytic activity still present after the addition of 100 μM BAU (Figure 2) (Liu et al., 1998, Cancer Res. 58:5418-5424).

The contribution of thymidine phosphorylase activity, another phosphorolytic enzyme with similar substrate specificity, to the BAU-insensitive phosphorolytic activity was evaluated using inhibitors of thymidine phosphorylase. More specifically, the addition of a specific thymidine phosphorylase inhibitor, 5-bromo-6-aminouracil (Liu et al., 1998, Cancer Res. 58:5418-5424), did not potentiate the inhibitory effect of BAU and approximately 30-35% of the initial phosphorolytic activity remained insensitive to classical uridine phosphorylase inhibitors.

As described in Liu et al. (1998, Cancer Res. 58:5418-5424), in order to confirm that the insensitivity to BAU was not due to the activity of TPase, the tissues

extract of a breast tumor was evaluated for both uridine and thymidine phosphorylase activities before being absorbed on a BAU affinity matrix. The activity of both enzymes was then determined in the flow-through in the presence of 100 μM of the respective inhibitor. The affinity column extracted approximately 80% of the UPase activity and the addition of BAU to the eluate marginally reduced the residual activity. None of the initial thymidine phosphorylase activity remained on the column and 100 μM 5-bromo-6-aminouracil caused a 75% reduction of the enzymatic capacity confirming that thymidine phosphorylase does not significantly contribute to the BAU-insensitive phosphorolytic activity present in breast tumor tissues.

The BAU-insensitive phosphorolytic activity in selected tumors, coupled with the potent inhibitory activity of BAU against the "classical" (*i.e.*, normal or wild type) UPase in normal human tissues, provides the rationale for combining BAU with 5-fluorouracil in the treatment of breast, head-neck, and ovarian tumors, among other diseases, disorders or conditions. Therefore, the data disclosed herein demonstrating, for the first time, that UPase in certain tumors is resistant to BAU inhibition has important implications in the development of diagnostics and therapeutics relating to various disorders, conditions and diseases, including tumors (*e.g.*, breast, head-neck, and ovarian tumors, among others) since the present invention discloses, for the first time, that UPI-resistant UPase is associated with and/or mediates tumorgenesis such that tumor, and cancer, development can be assessed using the methods of the present invention based on detection of UPI-resistant UPase in a tissue.

Example 2: Identification and characterization of mutations located in the nucleic acid encoding BAU-resistant mutant UPase

A number of different approaches were employed to identify the source of the novel BAU-insensitive phosphorolytic activity present in human breast cancers. It was previously postulated that the alternative activity was linked to a UPase-like gene and therefore various cDNA libraries were screened using various techniques. However, the data disclosed herein demonstrate that the only sequence that we were able to "fish out"

corresponded to the normal human UPase. Subsequently, the possible existence of UPase isoforms derived from the alternative splicing of the parent gene was determined. Eventually, as demonstrated by the data disclosed herein, it was established, for the first time, that the new activity was due to the presence of mutated forms of UPase. To further identify the mutant UPase(s), two different approaches were used both resulting in the isolation of novel, different UPase mutations.

In one case, a human breast cell line, MDA-MB-453 (ATCC No. HTB-131) that presents at least 50% of its phosphorolytic activity being insensitive to BAU inhibition, was used as a source for the isolation of BAU-resistant UPase. To screen for the presence of UPase mutants, total RNA was isolated from the breast cell line and the UPase cDNA was amplified by reverse transcriptase-polymerase chain reaction analysis (RT-PCR) (GIBCO) with a pair of primers, named HUP1 (GTGAAGTTTGTGTGTGTTGG, forward primer [SEQ ID NO:3]) and HUP4 (GAAGTCCAAGGAGCACATGG, reverse primer [SEQ ID NO:4]), respectively, flanking both sides of the putative UPase catalytic site (amino acids from about residue number 107 through 122). In brief, a human UPase specific antisense primer HUP5, TGCTGGTACTCGCTGAGCAG (SEQ ID NO:5), was used to synthesize the first strand of cDNA in the presence of 20 μl reverse transcription reaction mix containing 5 μg of total RNA, 5 pmol HUP5 primer, 1 μl Superscript transcriptase, and other complements as suggested and provided by the manufacturer. PCR reactions were conducted with the Ready-to-Go PCR beads (Pharmacia Biotech, Piscataway, NJ) per the manufacturer's instructions.

DNA sequencing data, provided by the Protein and Nucleic Acid Chemistry Facility of the Yale Cancer Center, showed a point mutation present in its active site (G-A) (Table 1), causing a GAG codon for glutamic acid to change into a AAG codon for lysine. No mutations were detected in the UPase sequence of MCF-7 cells (ATCC No. HTB-22) that are sensitive to BAU inhibition. Without wishing to be bound by any particular theory, the obvious change in the amino acid residue suggests that this mutation may result in the alteration of stereo structure at the enzyme active site

and be potentially responsible for the altered BAU sensitivity.

This mutation would also result in the disappearance of a SacI restriction site (GAGCTC), which can be exploited to screen for mutations in human breast cancer clinical specimens as well as in specimens from, *inter alia*, head-neck and ovarian tumors, and the like. The presence of such mutant can now be determined simply determined by SacI digestion of the RT-PCR products and agarose gel separation (Figure 3).

Southern blot hybridization was used to further confirm the PCR accuracy. Briefly, total RNA was extracted from human breast cancer tissues and cell lines, and reverse transcription and PCR amplification were performed as described above. 10 μl of PCR products were digested by 20 U SacI in 20 μl overnight, and then separated on 1.2% agarose gel. Using 0.5 N NaOH as a buffer, the DNA fragments were blotted on Nylon membranes (Amersham Life Sciences, Arlington Heights, IL) and probed with human UPase cDNA labeled with ^{32}P by random primer labeling kit (Amersham).

The data disclosed herein demonstrate that mutant UPase PCR products can not be cut by SacI and show only a 500 basepair (bp) band, on the contrary, the wild type UPase PCR products are cut by SacI and exhibit two bands of approximately 200 and 300 bps, respectively (Figure 3). All the bands hybridized with human UPase cDNA probe. This particular mutation was found in the human breast cell line MDA-MB-453 and two of five breast cancer tissues analyzed using this method and that presented at the initial biochemical characterization at least a 30% residual phosphorolytic activity in the presence of 100 μM BAU.

Table 1
Summary of mutations in human Breast Tumors

								SacI															
Normal UPase	701	ATCATGTTGCATGAGCTCATAAAGCTGCTGTACTATGCCCGGTGCTCCAACGTCACTATC	760																				
MCF-7				I	M	L	H	E	L	I	K	L	L	Y	Y	A	R	C	S	N	V	T	I
MDA-MB-453	701	ATCATGTTGCATAAGCTCATAAAGCTGCTGTACTATGCCCGGTGCTCCAACGTCACTATC	760																				
MDA-MB-468				I	M	L	H	K	L	I	K	L	L	Y	Y	A	R	C	S	N	V	T	I
Tumor 3-6																							
Tumor 1-8	701	ATCATGTTGCATGAGCTCATAAAGCTGCTGTACTATGCCCGGTGCTCCAACATCACTATC	760																				
				I	M	L	H	E	L	I	K	L	L	Y	Y	A	R	C	S	N	I	T	I

Alternatively, PCR-SSCP (polymerase chain reaction-single strand conformation polymorphism) was used to evaluate the same breast tumor tissues, *i.e.*, the breast cancer cell line MCF-7, which exhibits BAU-sensitivity, and also MDA-MB-468 (ATCC No. HTB-132) cells that were recently found insensitive to BAU, for mutations.

RNA was isolated from human breast cancer tissues or cells using TRIzol reagent (GIBCO/BRL, Gaithersburg, MD). The total RNA (1 μg) was treated with RNase-free DNase (1 U). cDNA was synthesized from 1 μg of total RNA. Reverse transcription was performed in a 20 μl volume of 1 X first strand buffer, 10 mM DTT, 500 μM of dNTPs, 15 mg/ml oligo(dT) primers, and 200 units of SuperScript II reverse transcriptase. The sample were first denatured for 1 minute at 70°C, then incubated at 42°C for 50 minutes. One μl of cDNA was used for PCR amplification. The oligonucleotide primers for UPase gene were radiolabeled ([r-ATP]^{32}P) with T4 polynucleotide kinase. The PCR was performed in a 5 μl volume for 35 cycles of 94°C for 1 minute, 55°C for 1 minute and 72°C 1.5 minutes. The PCR products were then mixed with 45 μl buffer containing 95% formamide, 20 mM EDTA, 0.05% bromophenol blue, and 0.05% xylene cyanol FF, loaded onto an 8% polyacrylamide gel (29:1 ratio of acrylamide to bis acrylamide) and separated at 4°C and room temperature, respectively at 2 watts.

The gel was dried on a filter paper with a vacuum gel dryer and exposed to X-ray film at -70°C for 6 to 12 hr with an intensifying screen. Cell lines and tumor samples containing mutations of the UPase gene (as revealed by a shift in mobility of the mutated exon 6 in the SSCP gel) and negative controls, including MCF-7 and paired normal tissues, were re-amplified by PCR. The PCR products were purified by separation on 0.8% Agarose gel and directly auto-sequenced by the Protein and Nucleic Acid Chemistry Facility of the Yale Cancer Center. All the exons of the UPase gene were evaluated for possible band shift. Only exon 6 was shifted in some tumors compared to a normal control.

Point mutations of the human UPase gene were detected in one breast cancer cell line (1T), MDA-MB-468, and two breast cancer specimens but no shift in mobility was detected in normal tissue adjacent to the human breast cancer (1N), or in MCF-7 cells (Figure 5).

These mutations were confirmed by direct sequencing. The mutation in MDA-MB-468 cells is located at 713 bp, it is a transversion from G to A leading to an amino acid change from Glu to Lys, as found in MDA-MB-453 cells utilizing PCR-RFLP and reported above. The identical mutation present in two breast cancer specimens was located at 752 bp, it was also a transversion from G to A, leading to an amino acid change from Val to Ile compared with the amino acid sequence of normal wild type human UPase (*i.e.*, GenBank Acc. No. CAA62369: MAATGANAEK AESHNDCPVR LLNPNIAKMK EDILYHFNLT TSRHNFPALF GDVKFVCVGG SPSRMKAFIR CVGAELGLDC PGRDYPNICA GTDRYAMYKV GPVLSVSHGM GIPSISIMLH ELIKLLYYAR CSNVTIIRIG TSGGIGLEPG TVVITEQAVD TCFKAEFEQI VLGKRVIRKT DLNKKLVQEL LLCSAELSEF TTVVGNTMCT LDFYEGQGRL DGALCSYTEK DKQAYLEAAY AAGVRNIEME SSVFAAMCSA CGLQAAVVCV TLLNRLEGDQ ISSPRNVLSE YQQRPQRLVS YFIKKKLSKA; SEQ ID NO:2). Nucleic acids encoding UPase were obtained from the adjacent normal tissues to the two breast cancer tumors were also sequenced and the sequences were found to be normal.

Overall, out of ten tumor specimens analyzed for mutations at exon 6,

seven tumors presenting point mutations were characterized. Three tumors contained the 713 bp G to A mutation and four human tumor samples presented the 752 bp G to A mutation. No mutations were detected in paired adjacent normal tissue or other normal control specimens analyzed.

Although the independent identification of specific mutations in the phosphorylase gene was encouraging, causative correlation to the phenotype of BAU insensitivity was essential to corroborate the postulated mechanism of an altered BAU binding. The data disclosed herein demonstrate that these mutant sequences were cloned, the proteins encoded by the sequences were expressed, and kinetic characterization and evaluation of BAU sensitivity were performed.

Construction of mutant human UPase prokaryotic expression vectors and protein expression vectors and protein expression

For the mutant at 713 bp, a 49 bp forward primer (ATG GGC ATT CCT TCT ATC TCA ATC ATG TTG CAT AAG CTC ATA AAG CTG C; SEQ ID NO:6), has been synthesized by the Protein and Nucleic Acid Chemistry Facility of the Yale Cancer Center (New Haven, CT), in which a Bsm I restriction site (GCATTC) and a site-targeted mutation (G-A, underlined) are included. The reverse primer GAA GTC CAA GGT GCA CAT GGT GTT CC (SEQ ID NO:7) contains a StyI site (CCT/AT/AGG). Using normal Upase cDNA as a template, a PCR product with site-targeted mutation was produced, and was used to replace the corresponding part in the wild type UPase cDNA in pBlue/HUP at the BsmI and StyI sites. The derived clones were subjected to sequencing confirmation before the mutant human UPase cDNA codon region was inserted into the EcoRI and HindIII sites of a pQE expression vector to obtain pQE/Mut S.

For the mutant at 752 bp, the PCR product with site-targeted mutation has been produced with the forward primer, GCT GTA CTA TGC CCG GTG CTC CAA CAT CAC TAT CAT CC (SEQ ID NO:8), containing the site-targeted mutation (underlined) and the TatI site (wGTACw), and the same reverse primer as indicated

above. The subcloning, sequencing confirmation and expression vector insertion procedures were followed using above described protocol to produce pQE/Mut T. Wild-type human UPase was prepared in parallel (pQE/HUP) and was used as a control to monitor protein preparation and enzyme activity. The constructs (pQE/HUP, pQE/Mut S and pQE/Mut T) were introduced by electroporation into M15 cells (QIAGEN, Chatsworth, CA), which host pREP4 producing lac repressor protein to regulate the recombinant protein expression. Three clones from each construct were selected initially to verify the expression of UPase protein by Western Blot.

The cells from each selected human UPase wild type, Mutant S and Mutant T 4 clones were incubated in 15 ml LB media with 100 μg/ml ampicillin and 25 μg/ml kanamycin overnight at 37°C, and then transferred into 1 liter fresh LB. After the cells grew to log phase, UPase expression were induced by 1 mM/ml IPTG for 4 hours. Cells were collected using 5,000 rpm for 10 minutes at 4°C, and the cell pellets were saved and frozen at –20°C overnight.

The next day, the pellets were resuspended in 30 ml lysis buffer (50 mM NaH_2PO_4, 300 mM NaC1 and 10 mM imidazole). After the cells were incubated with 1 mg/ml lysozyme (final concentration) on ice for 30 minutes, the cells were lysed using a sonicator at 6 x 10 second bursts. Cell debris was removed at 100,000 x g for 30 minutes and the supernatants were collected and histidine tagged UPase fusion proteins were bound to the Ni-NTA resin at 4°C for 1 hour. Then, the binding resin was loaded on mini-columns and was washed using 30 ml wash buffer (50 mM NaH_2PO_4, 300 mM NaC1 and 20 mM imidizole, pH 8.0). Purified UPase proteins were eluted using in 4 x 0.5 ml of elution buffer (50 mM NaH_2PO_4, 300 mM NaC1 and 250 mM imidizole, pH 8.0). Salts and lower molecular weight molecules were removed by dialysis against 50 mM NaH_2PO_4 (pH 7.5) overnight. The UPase activity was assayed using a 100 μ1 mix containing 50 mM Tris, 100 μM potassium phosphate, 200 μM ^{3}H-uridine, and 100 ng protein and BAU sensitivity was detected in the presence of various BAU concentrations (5-100 μM). The data disclosed in Figure 4 indicate that UPase activity of the mutant protein present in the S clone is mostly insensitive to even 100 μM BAU; however, the

mutant T protein was only partially inhibited by BAU. These data demonstrate that the two mutations isolated in human breast specimens and cell lines are, indeed, responsible for the insensitivity to BAU reported previously.

A more detained kinetic analysis was performed using the mutant T that expresses only a reduced sensitivity to BAU. For the mutant S, which was the most insensitive to BAU and for which the response to BAU is disclosed elsewhere herein, more detailed kinetics must be performed due to a limited solubility in buffer unless in the presence of at least 100 μM uridine, thereby altering the determination of the kinetic parameters. The use of various detergents in the assay buffer should allow the proper evaluation of the kinetic characteristics of this more BAU insensitive mutant.

The data disclosed herein confirms various degrees of sensitivity to BAU of the two UPase mutants. Without wishing to be bound by any particular theory, the limited solubility of Mutant S, unless in the presence of uridine, indicates for this protein undergoes a conformational change due to the amino acid change, which conformational change, and limited solubility, are reversible upon the presence of the natural substrate in the catalytic site.

Mutant T possesses catalytic properties similar to the wild-type enzyme exhibiting better enzymatic activity in the presence of the tested substrates (Table 2). The kinetic properties very closely resemble those of the normal protein except when in the presence of uracil as a substrate, wherein there was a significant 5-fold advantage in the Vmax (Tables 3 and 4).

Table 2
Substrate Specificity of recombinant Human UPase and Mutant T

Uridine Phosphorylase Activity (nmol/mg.sec)		
Substrate	rHUPase	Mutant T
Uridine	19.2±1.3	28.3±0.5
Uracil	4.2±0.7	8.5±0.5
5-Fluorouracil	6.5±0.3	10.2±1.6
Thymidine	0.4±0.2	0.8±0.3

Table 3

Kinetic Parameters of recombinant Human UPase (wild-type)

	Km (μM)	Vmax (nmol/mg.sec)	Vmax/Km
Uridine	219.2±25.3	74.7±8.9	0.340
Phosphate	205.0±32.3	9.7±0.7	0.047
Uracil	178.4±18.5	11.0±2.4	0.062
5-Fluorouracil	61.7±11.8	14.5±0.8	0.235
Ribose-1-phosphate	75.4±7.3	7.1±0.2	0.094

Table 4

Kinetic Parameters of UPase Mutant T

	Km (μM)	Vmax (nmol/mg.sec)	Vmax/Km
Uridine	195.3±31.3	54.5±3.8	0.280
Phosphate	162.5±21.4	18.5±1.0	0.052
Uracil	153.7±24.2	59.6±4.0	0.388
5-Fluorouracil	68.9±13.2	21.1±1.3	0.306
Ribose-1-phosphate	42.2±6.7	3.8±0.2	0.090

SSCP analysis of the UPase gene detected a mobility shift of exon 1. The direct sequencing of exon 1 demonstrated the presence of two main mutations: a GγA transversion at 40 bp and a triple mutation GγC (178), TγG (182), GγA (186). Out of 21 tumor specimens examined (Table 5), fifteen (about greater than 70%) were identified as containing a mutation at exon 1. Of the 15 tumors presenting mutation(s) in exon 1, 11 of them (approximately 75%) were characterized by the triple mutation. Again, no mutations were detected in paired normal tissues and normal controls examined.

Table 5
Mutations present in UPase exon 1 from human breast tumors

Tissue #		Exon 1 Mutations
1	Tumor 1	C to T (40)
2	Tumor 2	---
3	Tumor 3	C to T (40)
4	Tumor 4	C to T (40)
5	Tumor 5	C to T (135)
6	Normal 5	---
7	Tumor 6	---
8	Tumor 19580	---
9	Normal 19580	---
10	Tumor 15847	---
11	Tumor 17134	C to G (178), A to C (182), C to T (186)
12	Tumor 16047	C to G (178), A to C (182), C to T (186)
13	Tumor 18267	C to T (40), C to G (178), A to C (182), C to T (186)
14	Tumor 22832	---
15	Tumor 23542	C to G (178), A to C (182), C to T (186)
16	Tumor 2741	C to G (178), A to C (182), C to T (186)
17	Tumor 1459	C to G (178), A to C (182), C to T (186)
18	Tumor 2857	C to G (178), A to C (182), C to T (186)
19	Tumor 2352	C to G (178), A to C (182), C to T (186)
20	Tumor 1765	C to G (178), A to C (182), C to T (186)
21	Tumor 3266	---
22	Tumor 1535	C to G (178), A to C (182), C to T (186)
23	Tumor 468	---
24	Control 1	---
25	Control 2	---

Tissue #		Exon 1 Mutations
26	Control 3	---
27	Control 4	---
28	Control 5	---
29	Control 6	---
30	Control 7	---

Example 3: Characterization of additional mutations in the nucleic acid encoding UPase

Although the techniques disclosed previously elsewhere herein identified mutations in the nucleic acid encoding UPase that are associated with BAU-resistance and can be readily applied to identify additional mutations, other techniques are used to screen specimens in a rapid and secure fashion thereby detecting all the mutations present in UPase.

In order to identify in a rapid and systematic way all the mutations present that mediate and/or are associated with increased resistance to UPIs, several technologies are evaluated, including those which are commercially available through Third Wave Technology, Inc. (Madison, WI). These technologies have been shown to be successful in detecting mutations in other cancer related genes, such as BRCA1 and p53 (Eisinger et al., 1998, Cancer Res. 58:1588-1592; Rainaldi et al., 1998, Mutagenesis. 13:153-155).

In one aspect of the invention, a technology for detection of relevant mutations in UPase is CFLP (Cleavase Fragment Length Polymorphism). This technique is based on the ability of a structure specific nuclease (Cleavase) to recognize and cleave the junctions between single- and double-stranded regions of DNA (Lyamichev et al., 1993, Science. 260:778-783; Lieber, 1997, BioEssays 19:233-240).

Double-stranded DNA obtained by PCR when denaturated and cooled in low salt buffer tends to form secondary structures that consist of partially duplexed double-stranded hairpin regions intercalated with single-stranded regions. These

structures are unique to precise nucleotide sequences. The Cleavase enzyme recognizes and cuts at the 5'-region of the hairpin secondary structure yielding a series of fragments that, when separated on a polyacrylamide gel, generate unique fingerprints for different mutations or sequences.

This methodology allows the analysis of large fragments up to 1.5 kb as opposed to SSCP that currently is reliable only up to fragments of about 300 basepairs (bp). Furthermore, CFLP permits the isolation and sequencing of the individual fragments making a useful detection tool for the analysis of known mutations (by the fragment fingerprint), as well as for the identification and charaterization of novel variants. Such variants identified by the afore-mentioned techniques, and similar techniques known in the art or to be developed, are encompassed in the present invention.

Example 4: Further characterization of UPase mutants

Expression of inducible UPase mutants in knockout cells

To determine the role and functions of the UPase mutants in a whole cell system, uridine metabolism, 5-FU activation, "uridine rescue" and BAU effect are evaluated using tetracycline regulated expression of UPase mutants in nullified murine fibroblast cells obtained as described below.

Construction of tetracycline regulated UPase expression vector:

The plasmid pTRE (Clontech), containing the tetracycline-controlled transactivator (tTA) dependent promoter followed by a multicloning site is digested using EcoR I and Xba I. The mutant UPase cDNA encoding region, a sequencing confirmed-PCR product, is digested using the same restriction enzymes, *i.e.*, EcoR I and Xba I. The cDNA fragment is inserted into pTRE in the presence of 5 U of T4 DNA ligase in 20 μl overnight at 16°C to generate an inducible UPase expression vector designated pTRE-UPD. The plasmid DNA is amplified, isolated, and purified using QIAGEN columns. The plasmid DNA is stored at –20 °C till use.

Preparation of SV-40 immortalized murine UPase -/- and +/+ fibroblast cell lines:

An early stage primary fibroblast cell derived from day 12-13 UPase -/- and UPase +/+ mouse embryos is transfected with an expression vector encoding SV-40 early gene using calcium phosphate precipitation (*see, e.g.*, Sambrook et al., *supra*, and Ausubel et al., *supra*). Individual transformed colonies are isolated and the resulting immortalized cell lines are evaluated for the presence of SV-40 lg T antigen using, for example but not limited to, immunostaining techniques.

Establishment of cell lines that express UPase mutant protein(s) by tetracycline-controlled manner:

The SV-40 immortalized UPase -/- mouse fibroblast cells are co-transfected with plasmids pTRE-UPD and pTet-Off (Clontech) using, *e.g.*, calcium phosphate precipitation. The transfectants are selected by 200 μg/ml G418 in the media containing 0.1 μg/ml tetracycline, and then the UPase enzymatic activity as a result of the inducible UPase expression is assessed after removal of tetracycline. The positive clones with inducible UPase expression are collected for the further experiments and compared with SV-40 immortalized UPase +/+ cells which serve as a control (Holwell et al., 1007, J. Cell Sci. 110:1947-1956).

Example 5: Recombinant embryonic stem cells

Single and double UPase knockout embryonal stem cells (ES) were prepared using standard methods known in the art. A series of cellular pharmacology studies were conducted on selected single and double knockout embryonal stem cell clones. The data disclosed herein demonstrate that nullification of the uridine phosphorylase gene does not effect cell growth, size and the distribution of the ribonucleotide pools, nor does nullification contribute to any change in cell cycle distribution.

Exposure of the ES cells to increasing concentrations of N-(phosphonacetyl)-L-aspartic acid (PALA), a transitional stage inhibitor in the

condensation of the carbamylphosphate with L-aspartic acid, which is the first step in *de novo* pyrimidine synthesis, clearly demonstrated major differences in the contribution of the *de novo* pathway to cell proliferation among the different ES cell populations. Among the ES cell populations, wild-type control ES cells relied the most on the *de novo* pyrimidine synthesis demonstrating an ED_{50} of approximately 100 μM of PALA. The single knockout cells, that still maintain at least about 50% of the wild type phosphorolytic activity, were modestly sensitive to the inhibitor with about 20% inhibition at 200 μM PALA. Unlike the other ES cell populations, the double knockout cells are quite insensitive to this agent that directly impairs the *de novo* pyrimidine synthesis and the double knockout ES cells exhibited a ED_{50} 1 mM. These data suggest a major contribution of the pyrimidine salvage pathway in the absence of the catabolic activity of uridine phosphorylase. Uridine (50 μM) was able to rescue PALA antiproliferative activity in all ES clones.

The contribution of the pyrimidine salvage pathway was directly evaluated by determining the incorporation of tritiated uridine into the nucleic acids. The data disclosed herein demonstrate that the UPase double knockout ES cells have a 2-fold advantage over the wild type cell population in the utilization of uridine present in the medium (approximately 2 μM, a physiological concentration) (Table 6).

Table 6

Incorporation of [^{3}H]-Uridine into Nucleic Acids

Cell Type	nmol/10^6cells
Wild-type	1.25±0.14
Single Knockout	1.39±0.18
Double Knockout (clone)	2.45±0.08
Double Knockout (clone)	2.55±0.13

The analysis of thymidine phosphorylase activity, an enzyme that despite a lower efficiency shares substrate specificity with uridine phosphorylase, did not demonstrate any enzymatic change between knockout and wild type ES cells. Further,

data disclosed herein demonstrate that the uptake of uridine, both Na^+-dependent and facilitated diffusion, appear unchanged.

The contribution of uridine phosphorylase to the activation and anti-proliferative activity of 5-FU was evaluated in the various ES cell lines by incubating the cells with increasing concentrations of 5-FU for 72 hours. The data disclosed herein (*e.g.*, in Table 7) demonstrate that there is a substantial difference and a higher sensitivity to 5-FU for the ES wild type cells demonstrating the critical role of uridine phosphorylase and the salvage pathway to the activation of 5-FU to fluorouridine and, eventually, to fluoronucleotides using this cell model. The single knockout cells exhibited an intermediate sensitivity to 5-FU, further confirming the role of uridine phosphorylase in protecting cells from the toxic effects of 5-FU. The data disclosed herein further demonstrate that shorter exposures of about 4 and 24 hours to the anticancer agent, less clearly differentiates the sensitivity of wild-type ES cells from the double knockout populations.

Table 7

Sensitivity of ES cells differently expressing UPase to various anticancer agents

Drug Treatment	ES Parent	ES Single KO	ES Double KO (16)	ES Double KO (22)
PALA	75	200	1,000	1,000
PALA+Urd	2,000	>2,000	>2,000	>2,000
5-FU	0.3	0.6	2.5	2.5
5-FU+Urd	*0.7*	*1.2*	*2*	*2*
FUrd	0.060	0.070	0.080	0.080
FUrd+Urd	*0.3*	*0.6*	*0.6*	*0.6*
FdUrd	0.020	0.020	0.020	0.020
AraC	0.25	0.25	0.25	0.25
Doxorubicin	0.02	0.03	0.03	0.03

Exposure of the different ES cells to other fluoropyrimidines that do not require UPase or orotate phosphoribosyltransferase (OPRTase) for their initial activation, such as, but not limited to, 5-fluorouridine and 5-fluorodeoxyuridine, demonstrated that there was no significant difference in sensitivity among the cell types. Furthermore,

incubation with another nucleoside analog, ara-C, requiring activation via deoxycytidine kinase, did not demonstrate any difference in inhibition among the cell types.

The data disclosed herein suggest that lack of UPase activity does not affect the size of the other pyrimidine nucleotide pool, and this was further confirmed using direct measurement of the ribonucleotide pools.

The data disclosed herein further demonstrate that incubation with doxorubicin, another anticancer agent that does not share any target or mechanism of activation with the previously disclosed drugs, indicate that there is no difference among the four ES cell populations in the response to this agent.

The disclosures of each and every patent, patent application, and publication cited herein are hereby incorporated herein by reference in their entirety.

While the invention has been disclosed with reference to specific embodiments, it is apparent that other embodiments and variations of this invention may be devised by others skilled in the art without departing from the true spirit and scope of the invention. The appended claims are intended to be construed to include all such embodiments and equivalent variations.

CLAIMS

What is claimed is:

1. An isolated nucleic acid encoding a human uridine phosphorylase wherein said uridine phosphorylase is resistant to a uridine phosphorylase inhibitor.

2. The nucleic acid of claim 1, wherein said uridine phosphorylase inhibitor is selected from the group consisting of benzylacyclouridine, benzyloxybenzylacyclouridine, aminomethyl-benzylacyclouridine, aminomethyl-benzyloxybenzylacyclouridine, hydroxymethyl-benzylacyclouridine, 5-(phenylselenyl)acyclouridine, 2',3',5'-tri-O-acetyluridine, 1-[(2-hydroxyethoxy)methyl]-5-phenylthiouracil, aryl-substituted 5-benzyluracils, 1-[(2-hydroxyethoxy)methyl]-5-benzyluracils, aryl-substituted 1-((2-hydroxyethoxy)methyl)-5-(3-phenoxybenzyl)uracil, 5-(benzyloxybenzyl)barbituric acid acyclonucleoside, and hydroxymethyl-benzyloxybenzylacyclouridine.

3. An isolated nucleic acid encoding a human uridine phosphorylase wherein said uridine phosphorylase is resistant to a uridine phosphorylase inhibitor and further wherein the sequence of said nucleic acid comprises at least one mutation selected from the group consisting of a mutation located in exon 1 and a mutation located in exon 6.

4. An isolated nucleic acid encoding a human uridine phosphorylase, wherein said uridine phosphorylase is resistant to a uridine phosphorylase inhibitor and further wherein the sequence of said nucleic acid comprises at least one mutation selected from the group consisting of a C to T at nucleotide 40, a C to T at nucleotide 135, a C to G at nucleotide 178, an A to C at nucleotide 182, a C to T at nucleotide 186, a G to A at nucleotide 713, and a G to A at nucleotide 752.

5. The nucleic acid of claim 4, wherein said uridine phosphorylase inhibitor is selected from the group consisting of benzylacyclouridine, benzyloxybenzylacyclouridine, aminomethyl-benzylacyclouridine, aminomethyl-benzyloxybenzylacyclouridine, hydroxymethyl-benzylacyclouridine, 5-(phenylselenyl)acyclouridine, 2',3',5'-tri-O-acetyluridine, 1-[(2-hydroxyethoxy)methyl]-5-phenylthiouracil, aryl-substituted 5-benzyluracils, 1-[(2-hydroxyethoxy)methyl]-5-benzyluracils, aryl-substituted 1-((2-hydroxyethoxy)methyl)-5-(3-phenoxybenzyl)uracil, 5-(benzyloxybenzyl)barbituric acid acyclonucleoside, and hydroxymethyl-benzyloxybenzylacyclouridine.

6. An isolated human uridine phosphorylase polypeptide, wherein said polypeptide is resistant to a uridine phosphorylase inhibitor.

7. An isolated human uridine phosphorylase polypeptide, wherein said polypeptide is resistant to a uridine phosphorylase inhibitor and further wherein said polypeptide is encoded by an isolated nucleic acid comprising at least one mutation selected from the group consisting of a mutation located in exon 1 and a mutation located in exon 6.

8. An isolated human uridine phosphorylase polypeptide, wherein said polypeptide is resistant to a uridine phosphorylase inhibitor and further wherein said polypeptide is encoded by an isolated nucleic acid comprising at least one mutation selected from the group consisting of a change from a C to T at nucleotide 40, a C to T at nucleotide 135, a C to G at nucleotide 178, an A to C at nucleotide 182, a C to T at nucleotide 186, a G to A at nucleotide 713, and a G to A at nucleotide 752.

9. The polypeptide of claim 8, wherein said uridine phosphorylase inhibitor is selected from the group consisting of benzylacyclouridine,

benzyloxybenzylacyclouridine, aminomethyl-benzylacyclouridine, aminomethyl-benzyloxybenzylacyclouridine, hydroxymethyl-benzylacyclouridine, 5-(phenylselenyl)acyclouridine, 2',3',5'-tri-O-acetyluridine, 1-[(2-hydroxyethoxy)methyl]-5-phenylthiouracil, aryl-substituted 5-benzyluracils, 1-[(2-hydroxyethoxy)methyl]-5-benzyluracils, aryl-substituted 1-((2-hydroxyethoxy)methyl)-5-(3-phenoxybenzyl)uracil, 5-(benzyloxybenzyl)barbituric acid acyclonucleoside, and hydroxymethyl-benzyloxybenzylacyclouridine.

10. An isolated human uridine phosphorylase polypeptide, wherein said polypeptide is resistant to a uridine phosphorylase inhibitor and further wherein the amino acid sequence of said polypeptide comprises at least one mutation selected from the group consisting of a change from glutamic acid to lysine at amino acid residue number 121, and a change from valine to isoleucine at amino acid residue number 134.

11. An antibody that specifically binds with the isolated polypeptide of claim 10, or a fragment thereof.

12. An antibody that specifically binds with the isolated polypeptide of claim 8, or a fragment thereof.

13. The isolated nucleic acid of claim 1, said nucleic acid further comprising a nucleic acid encoding a tag polypeptide covalently linked thereto.

14. The isolated nucleic acid of claim 13, wherein said tag polypeptide is selected from the group consisting of a myc tag polypeptide, a glutathione-S-transferase tag polypeptide, a green fluorescent protein tag polypeptide, a myc-pyruvate kinase tag polypeptide, a His6 tag polypeptide, an influenza virus hemagglutinin tag polypeptide, and a maltose binding protein tag polypeptide.

15. The isolated nucleic acid of claim 4, said nucleic acid further comprising a nucleic acid encoding a tag polypeptide covalently linked thereto.

16. The isolated nucleic acid of claim 15, wherein said tag polypeptide is selected from the group consisting of a myc tag polypeptide, a glutathione-S-transferase tag polypeptide, a green fluorescent protein tag polypeptide, a myc-pyruvate kinase tag polypeptide, a His6 tag polypeptide, an influenza virus hemagglutinin tag polypeptide, and a maltose binding protein tag polypeptide.

17. The isolated nucleic acid of claim 1, said nucleic acid further comprising a nucleic acid encoding a promoter/regulatory sequence operably linked thereto.

18. A vector comprising the nucleic acid of claim 1.

19. A recombinant cell comprising the isolated nucleic acid of claim 1.

20. The isolated nucleic acid of claim 4, said nucleic acid further comprising a nucleic acid encoding a promoter/regulatory sequence operably linked thereto.

21. A vector comprising the nucleic acid of claim 4.

22. A recombinant cell comprising the isolated nucleic acid of claim 4.

23. A transgenic non-human mammal comprising the isolated nucleic acid of claim 1.

24. A transgenic non-human mammal comprising the isolated nucleic acid of claim 4.

25. An isolated nucleic acid that hybridizes with high stringency with a nucleic acid encoding human uridine phosphorylase, wherein said isolated nucleic acid is selected from the group consisting of a nucleic acid having the sequence SEQ ID NO:3, a nucleic acid having the sequence SEQ ID NO:4, a nucleic acid having the sequence SEQ ID NO:5, a nucleic acid having the sequence SEQ ID NO:6, a nucleic acid having the sequence SEQ ID NO:7, and a nucleic acid having the sequence SEQ ID NO:8.

26. A method for detecting the presence or absence of a nucleic acid encoding a human uridine phosphorylase resistant to a uridine phosphorylase inhibitor, said method comprising

(a) contacting a sample with a nucleic acid probe or primer which specifically hybridizes with said nucleic acid; and

(b) determining whether said nucleic acid probe or primer binds with a nucleic acid in said sample whereby when said nucleic acid probe or primer binds with a nucleic acid in said sample, said sample contains a nucleic acid which specifically hybridizes with said nucleic acid,

thereby detecting the presence or absence of said nucleic acid in a sample .

27. The method of claim 26, wherein said nucleic acid probe or primer is selected from the group consisting of a nucleic acid having the sequence SEQ ID NO:3, a nucleic acid having the sequence SEQ ID NO:4, a nucleic acid having the sequence SEQ ID NO:5, a nucleic acid having the sequence SEQ ID NO:6, a nucleic acid having the sequence SEQ ID NO:7, and a nucleic acid having the sequence SEQ ID NO:8.

28. The method of claim 26, wherein said sample comprises mRNA molecules.

29. A method for detecting the presence or absence of a nucleic acid encoding a human uridine phosphorylase resistant to a uridine phosphorylase inhibitor in a biological sample, said method comprising

(a) amplifying a nucleic acid encoding a human uridine phosphorylase present in a biological sample wherein said nucleic acid comprises at least one mutation selected from the group consisting of a mutation located in exon 1 and a mutation located in exon 6;

(b) detecting any amplified target nucleic acid formed in (a) whereby when said nucleic acid is amplified said biological sample contains said nucleic acid which encodes a human uridine phosphorylase resistant to a uridine phosphorylase inhibitor,

thereby detecting the presence or absence of a nucleic acid encoding a human uridine phosphorylase resistant to a uridine phosphorylase inhibitor in a biological sample.

30. The method of claim 29, wherein said mutation in exon 1 is selected from the group consisting of a change from a C to T at nucleotide 40, a C to T at nucleotide 135, a C to G at nucleotide 178, an A to C at nucleotide 182, and a C to T at nucleotide 186.

31. The method of claim 29, wherein said mutation in exon 6 is selected from the group consisting of a change from G to A at nucleotide 713, and a change from G to A at nucleotide 752.

32. A kit for detecting a nucleic acid encoding human uridine phosphorylase resistant to a uridine phosphorylase inhibitor, said kit comprising at least one nucleic acid selected from the group consisting of a nucleic acid having the sequence SEQ ID NO:3, a nucleic acid having the sequence SEQ ID NO:4, a nucleic acid having

the sequence SEQ ID NO:5, a nucleic acid having the sequence SEQ ID NO:6, a nucleic acid having the sequence SEQ ID NO:7, and a nucleic acid having the sequence SEQ ID NO:8, and an instructional material for the use thereof.

33. A method of detecting a cancer in a human, said method comprising obtaining a biological sample from said human, detecting the presence or absence of uridine phosphorylase resistant to a uridine phosphorylase inhibitor, wherein the presence of uridine phosphorylase resistant to a uridine phosphorylase inhibitor in said sample is an indication that said vertebrate animal has a cancer, thereby detecting a cancer in said human.

34. The method of claim 33, wherein said cancer is selected from the group consisting of breast cancer, head-neck cancer, and ovarian cancer.

35. The method of claim 33, wherein said biological sample is selected from the group consisting of a breast tissue sample, a head-neck tissue sample, and an ovarian tissue sample.

36. The method of claim 33, wherein said detection is assessed using a method selected from the group consisting of a method of detecting uridine phosphorylase activity resistant to a uridine phosphorylase inhibitor and a method of detecting a mutation in a nucleic acid encoding uridine phosphorylase wherein said mutation is associated with uridine phosphorylase resistance to a uridine phosphorylase inhibitor.

37. The method of claim 36, wherein said mutation is selected from a mutation in exon 1 and a mutation in exon 6.

38. The method of claim 37, wherein said mutation in exon 1 is selected from the group consisting of a change from a C to T at nucleotide 40, a C to T at nucleotide 135, a C to G at nucleotide 178, an A to C at nucleotide 182, and a C to T at nucleotide 186.

39. The method of claim 37, wherein said mutation in exon 6 is selected from the group consisting of a change from G to A at nucleotide 713, and a change from G to A at nucleotide 752.

40. The method of claim 39, wherein said mutation is detected using a nucleic acid selected from the group consisting of a nucleic acid having the sequence SEQ ID NO:3, a nucleic acid having the sequence SEQ ID NO:4, a nucleic acid having the sequence SEQ ID NO:5, a nucleic acid having the sequence SEQ ID NO:6, a nucleic acid having the sequence SEQ ID NO:7, and a nucleic acid having the sequence SEQ ID NO:8.

41. A method of monitoring the treatment of a human previously diagnosed with cancer, said method comprising:

(a) assessing the level of uridine phosphorylase resistant to a uridine phosphorylase inhibitor in a first biological sample obtained from said human to determine an initial level of uridine phosphorylase resistant to a uridine phosphorylase inhibitor;

(b) administering an anti-cancer therapy to said human;

(c) assessing the level of uridine phosphorylase resistant to a uridine phosphorylase inhibitor in a second otherwise identical biological sample obtained from said human during or after said therapy;

(d) comparing said level of uridine phosphorylase resistant to a uridine phosphorylase inhibitor in said first biological sample with said level of uridine phosphorylase resistant to a uridine phosphorylase inhibitor in said second biological

sample; and

(e) correlating any reduction in level of uridine phosphorylase resistant to a uridine phosphorylase inhibitor with the effectiveness of said anti-cancer therapy,

thereby monitoring the treatment of a human previously diagnosed with cancer.

42. The method of claim 41, said method further comprising repeating (b) through (e) during the course of said human's illness, anti-cancer therapy, or any period or portion thereof.

43. The method of claim 41, wherein said level of uridine phosphorylase activity resistant to a uridine phosphorylase inhibitor is assessed using a method selected from the group consisting of a method of detecting uridine phosphorylase activity resistant to a uridine phosphorylase inhibitor and a method of detecting a mutation in a nucleic acid encoding uridine phosphorylase wherein said mutation is associated with uridine phosphorylase resistance to a uridine phosphorylase inhibitor.

44. A method of identifying a human afflicted with a disease, disorder or condition associated with expression of uridine phosphorylase resistant to a uridine phosphorylase inhibitor, said method comprising detecting a mutation in a nucleic acid encoding uridine phosphorylase in a human associated with resistance to a uridine phosphorylase inhibitor, thereby detecting a human afflicted with a disease, disorder or condition associated with expression of uridine phosphorylase resistant to a uridine phosphorylase inhibitor.

45. A method of detecting a mutation in a uridine phosphorylase allele in a human, said method comprising comparing the nucleic acid sequence encoding uridine phosphorylase resistant to a uridine phosphorylase inhibitor obtained from a tumor tissue

sample obtained from a human with the nucleic acid sequence encoding uridine phosphorylase not resistant to a uridine phosphorylase inhibitor obtained from an otherwise identical non-tumor tissue sample obtained from said human, wherein any difference between said nucleic acid sequence encoding uridine phosphorylase resistant to a uridine phosphorylase inhibitor and said sequence encoding uridine phosphorylase not resistant to a uridine phosphorylase inhibitor detects a mutation in a uridine phosphorylase allele in said human.

46. A method of detecting a mutation in a uridine phosphorylase allele in a human, said method comprising comparing the genomic nucleic acid sequence encoding uridine phosphorylase resistant to a uridine phosphorylase inhibitor with the genomic nucleic acid sequence encoding uridine phosphorylase not resistant to a uridine phosphorylase inhibitor, wherein any difference between said genomic nucleic acid sequence encoding uridine phosphorylase resistant to a uridine phosphorylase inhibitor and said genomic nucleic acid sequence encoding uridine phosphorylase not resistant to a uridine phosphorylase inhibitor detects a mutation in a uridine phosphorylase allele in said human.

47. A method of treating cancer in a human receiving 5-fluorouracil, said method comprising assessing the presence of uridine phosphorylase resistant to a uridine phosphorylase inhibitor in a tumor sample obtained from a human where the presence of uridine phosphorylase resistant to a uridine phosphorylase is an indication that said uridine phosphorylase inhibitor should be administered to said human, thereby treating a cancer in a human receiving 5-fluorouracil.

48. A kit for detecting a cancer in a human, said kit comprising at least one nucleic acid selected from the group consisting of a nucleic acid having the sequence SEQ ID NO:3, a nucleic acid having the sequence SEQ ID NO:4, a nucleic acid having the sequence SEQ ID NO:5, a nucleic acid having the sequence SEQ ID NO:6, a nucleic

acid having the sequence SEQ ID NO:7, and a nucleic acid having the sequence SEQ ID NO:8, and an instructional material for the use thereof.

49. The kit of claim 48, wherein said cancer is selected from the group consisting of breast cancer, head-neck cancer, and ovarian cancer.

50. A kit for monitoring the treatment of a human previously diagnosed with cancer, said kit comprising at least one nucleic acid selected from the group consisting of a nucleic acid having the sequence SEQ ID NO:3, a nucleic acid having the sequence SEQ ID NO:4, a nucleic acid having the sequence SEQ ID NO:5, a nucleic acid having the sequence SEQ ID NO:6, a nucleic acid having the sequence SEQ ID NO:7, and a nucleic acid having the sequence SEQ ID NO:8, and an instructional material for the use thereof.

51. A kit for identifying a human afflicted with a disease, disorder or condition associated with expression of uridine phosphorylase resistant to a uridine phosphorylase inhibitor, said kit comprising at least one nucleic acid selected from the group consisting of a nucleic acid having the sequence SEQ ID NO:3, a nucleic acid having the sequence SEQ ID NO:4, a nucleic acid having the sequence SEQ ID NO:5, a nucleic acid having the sequence SEQ ID NO:6, a nucleic acid having the sequence SEQ ID NO:7, and a nucleic acid having the sequence SEQ ID NO:8, and an instructional material for the use thereof.

52. A kit for detecting a mutation in a uridine phosphorylase allele in a human, said kit comprising at least one nucleic acid selected from the group consisting of a nucleic acid having the sequence SEQ ID NO:3, a nucleic acid having the sequence SEQ ID NO:4, a nucleic acid having the sequence SEQ ID NO:5, a nucleic acid having the sequence SEQ ID NO:6, a nucleic acid having the sequence SEQ ID NO:7, and a

nucleic acid having the sequence SEQ ID NO:8, and an instructional material for the use thereof.

53. A kit for treating cancer in a human receiving 5-fluorouracil, said kit comprising at least one nucleic acid selected from the group consisting of a nucleic acid having the sequence SEQ ID NO:3, a nucleic acid having the sequence SEQ ID NO:4, a nucleic acid having the sequence SEQ ID NO:5, a nucleic acid having the sequence SEQ ID NO:6, a nucleic acid having the sequence SEQ ID NO:7, and a nucleic acid having the sequence SEQ ID NO:8, and an instructional material for the use thereof.

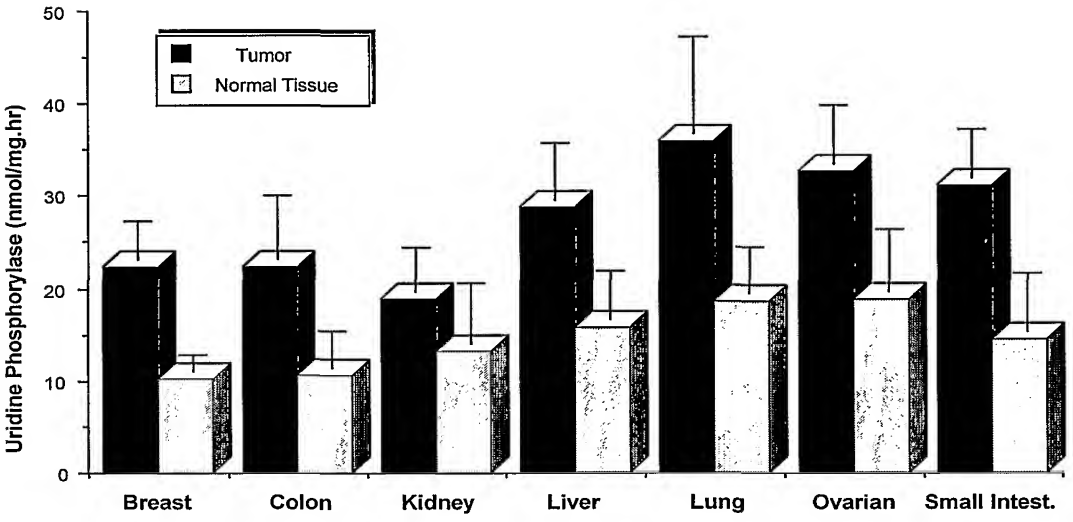
Tumor
Normal Tissue
Uridine Phosphorylase (nmol/mg.hr)
50
40
30
20
10
0
Breast
Colon
Kidney
Liver
Lung
Ovarian
Small Intest.

FIG. 1

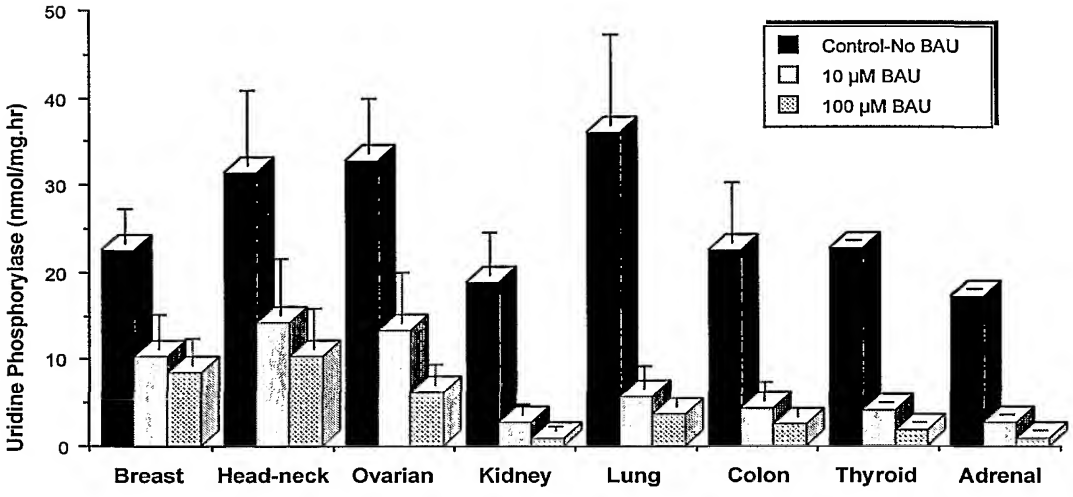
Uridine Phosphorylase (nmol/mg.hr)
50
40
30
20
10
0
Control-No BAU
10 µM BAU
100 µM BAU
Breast
Head-neck
Ovarian
Kidney
Lung
Colon
Thyroid
Adrenal

FIG. 2

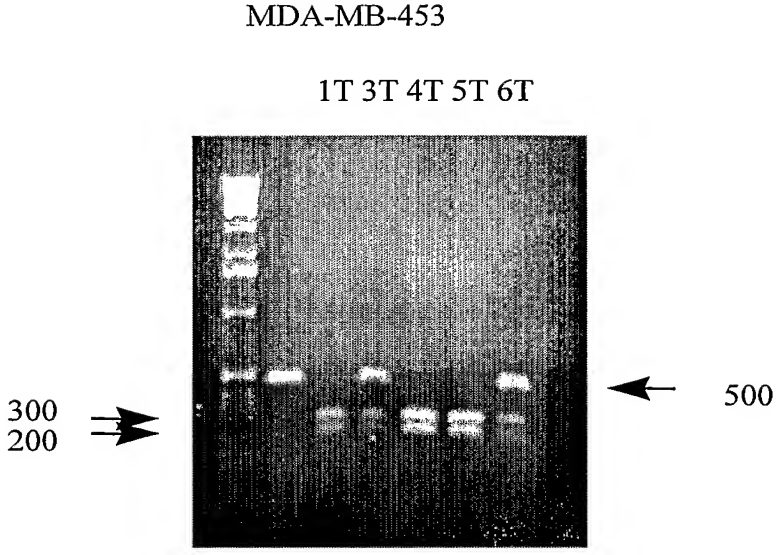

FIG. 3

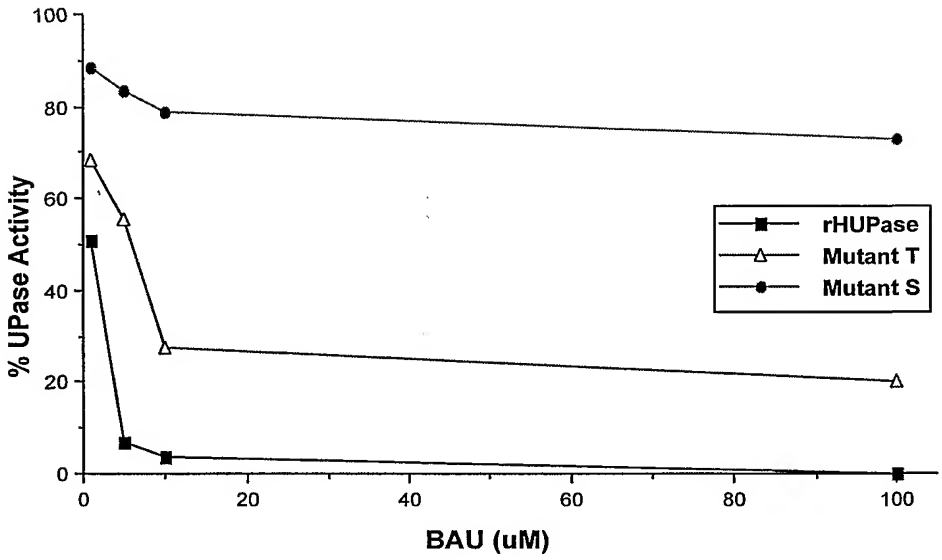
100
80
60
40
20
0
% UPase Activity
0
20
40
60
80
100
BAU (uM)
rHUPase
Mutant T
Mutant S

FIG. 4

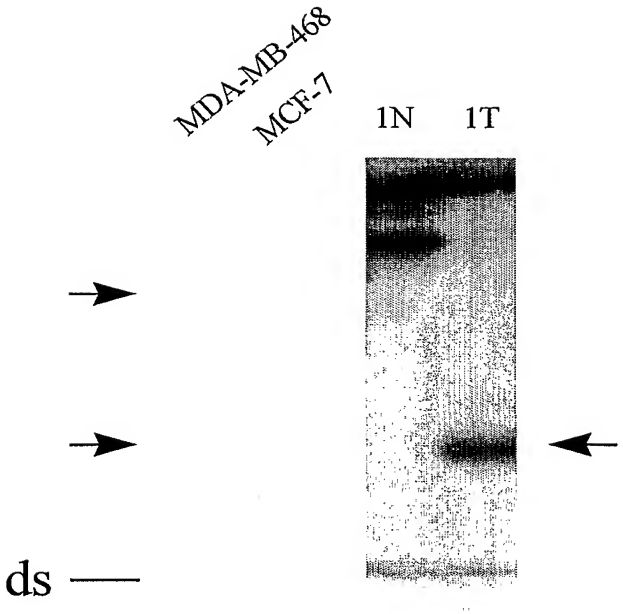

FIG. 5

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1    attcgagtgc tccggagaac agacccgcgc cccgccgtcc gcgagcctcc cgagagccgt
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121  cgcgggtgta gcgggctctct gcgcaggccg agtgggccca gagaagcgag gaactccgca
181  gatcgccgac acgtctcgtc tcctgtccca attcagggct tggtgaggtg actcgcggtc
241  gcgggtgact cgccggcagg acactgcctg gaacgcctgg agcgcctccc actgcagacg
301  tctgtccgcc tccagccgct ctcctctgac gggtcctgcc tcagttggcg gaatggcggc
361  cacgggagcc aatgcagaga aagctgaaag tcacaatgat tgccccgtca gacttttaaa
421  tccaaacata gcaaaaatga aagaagatat tctctatcat ttcaatctca ccactagcag
481  acacaatttc ccagccttgt ttggagatgt gaagtttgtg tgtgttggtg gaagcccctc
541  ccggatgaaa gccttcatca ggtgcgttgg tgcagagctg ggccttgact gcccaggtag
601  agactatccc aacatctgtg cgggaactga ccgctatgcc atgtataaag taggaccggt
661  gctgtctgtc agtcatggta tgggcattcc ttctatctca atcatgttgc atggagctcat
721  aaagctgctg tactatgccc ggtgctccaa cgtcactatc atccgcattg gcacttctgg
781  tgggataggt ctggagcccg gcactgtggt cataacagag caggcagtgg atacctgctt
841  caaggcagag tttgagcaga ttgtcctggg gaagcgggtc atccggaaaa cggaccttaa
901  caagaagctg gtgcaggagc tgttgctgtg ttctgcagag ctgagcgagt tcaccacagt
961  ggtggggaac accatgtgca ccttggactt ctatgaaggg caaggccgtc tggatggggc
1021 tctctgctcc tacacggaga aggacaagca ggcgtatctg gaggcagcct atgcagccgg
1081 cgtccgcaat atcgagatgg agtcctcggt gtttgccgcc atgtgcagcg cctgcggcct
1141 ccaagcggcc gtggtgtgtg tcaccctcct gaaccgcctg gaaggggacc agatcagcag
1201 ccctcgcaat gtgctcagcg agtaccagca gaggccgcag cggctggtga gctacttcat
1261 caagaagaaa ctgagcaagg cctgagcgct gccctgcacc tccgcagacc tgctgtgatg
1321 acttgccatt aaaagcattg tccaaaccc
```

SEQ I D NO:1
Nucleic acid sequence of human UPase (GenBank Acc. No. NM 003364)

FIG. 6

```
1   MAATGANAEK AESHNDCPVR LLNPNIAKMK EDILYHFNLT TSRHNFPALF GDVKFVCVGG
61  SPSRMKAFIR CVGAELGLDC PGRDYPNICA GTDRYAMYKV GPVLSVSHGM GIPSISIMLH
121 ELIKLLYYAR CSNVTIIRIG TSGGIGLEPG TVVITEQAVD TCFKAEFEQI VLGKRVIRKT
181 DLNKKLVQEL LLCSAELSEF TTVVGNTMCT LDFYEGQGRL DGALCSYTEK DKQAYLEAAY
241 AAGVRNIEME SSVFAAMCSA CGLQAAVVCV TLLNRLEGDQ ISSPRNVLSE YQQRPQRLVS
301 YFIKKKLSKA
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SEQ I D NO:2
Amino acid sequence of human UPase (GenBank Acc. No. CAA62369)

FIG. 7

SEQUENCE LISTING

<110> YALE UNIVERSITY
PIZZORNO, Giuseppe
CAO, Deliang
ZHANG, Dekai

<120> COMPOSITIONS, METHODS AND KITS RELATING TO URIDINE PHOSPHORYLASE GENE MUTATIONS

<130> 44574-5085-WO

<140>
<141>

<150> US 60/182,273
<151> 2000-02-14

<160> 8

<170> PatentIn Ver. 2.1

<210> 1
<211> 1349
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (353)..(1285)

<220>
<221> mutation
<222> (40)..(752)
<223> DNA sequence: y at positions 40, 135 and 186 = c or t; s at position 178 = c or g; m at position 182 = a or c; r at positions 713 and 752 = a or g. Protein sequence: Xaa at position 121 = Glu or Lys; Xaa at position 134 = Val or Ile.

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cgcgggtgta gcggytctct gcgcaggccg agtgggccca gagaagcgag gaactccsca 180

gmtcgycgac acgtctcgtc tcctgtccca attcagggct tggtgaggtg actcgcggtc 240

gcgggtgact cgccggcagg acactgcctg gaacgcctgg agcgcctccc actgcagacg 300

tctgtccgcc tccagccgct ctcctctgac gggtcctgcc tcagttggcg ga atg gcg 358
Met Ala
1

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gcc acg gga gcc aat gca gag aaa gct gaa agt cac aat gat tgc ccc   406
Ala Thr Gly Ala Asn Ala Glu Lys Ala Glu Ser His Asn Asp Cys Pro
          5                  10                  15

gtc aga ctt tta aat cca aac ata gca aaa atg aaa gaa gat att ctc   454
Val Arg Leu Leu Asn Pro Asn Ile Ala Lys Met Lys Glu Asp Ile Leu
     20                  25                  30

tat cat ttc aat ctc acc act agc aga cac aat ttc cca gcc ttg ttt   502
Tyr His Phe Asn Leu Thr Thr Ser Arg His Asn Phe Pro Ala Leu Phe
 35                  40                  45                  50

gga gat gtg aag ttt gtg tgt gtt ggt gga agc ccc tcc cgg atg aaa   550
Gly Asp Val Lys Phe Val Cys Val Gly Gly Ser Pro Ser Arg Met Lys
                 55                  60                  65

gcc ttc atc agg tgc gtt ggt gca gag ctg ggc ctt gac tgc cca ggt   598
Ala Phe Ile Arg Cys Val Gly Ala Glu Leu Gly Leu Asp Cys Pro Gly
             70                  75                  80

aga gac tat ccc aac atc tgt gcg gga act gac cgc tat gcc atg tat   646
Arg Asp Tyr Pro Asn Ile Cys Ala Gly Thr Asp Arg Tyr Ala Met Tyr
         85                  90                  95

aaa gta gga ccg gtg ctg tct gtc agt cat ggt atg ggc att cct tct   694
Lys Val Gly Pro Val Leu Ser Val Ser His Gly Met Gly Ile Pro Ser
    100                 105                 110

atc tca atc atg ttg cat rag ctc ata aag ctg ctg tac tat gcc cgg   742
Ile Ser Ile Met Leu His Xaa Leu Ile Lys Leu Leu Tyr Tyr Ala Arg
115                 120                 125                 130

tgc tcc aac rtc act atc atc cgc att ggc act tct ggt ggg ata ggt   790
Cys Ser Asn Xaa Thr Ile Ile Arg Ile Gly Thr Ser Gly Gly Ile Gly
                135                 140                 145

ctg gag ccc ggc act gtg gtc ata aca gag cag gca gtg gat acc tgc   838
Leu Glu Pro Gly Thr Val Val Ile Thr Glu Gln Ala Val Asp Thr Cys
            150                 155                 160

ttc aag gca gag ttt gag cag att gtc ctg ggg aag cgg gtc atc cgg   886
Phe Lys Ala Glu Phe Glu Gln Ile Val Leu Gly Lys Arg Val Ile Arg
        165                 170                 175

aaa acg gac ctt aac aag aag ctg gtg cag gag ctg ttg ctg tgt tct   934
Lys Thr Asp Leu Asn Lys Lys Leu Val Gln Glu Leu Leu Leu Cys Ser
    180                 185                 190

gca gag ctg agc gag ttc acc aca gtg gtg ggg aac acc atg tgc acc   982
Ala Glu Leu Ser Glu Phe Thr Thr Val Val Gly Asn Thr Met Cys Thr
195                 200                 205                 210

ttg gac ttc tat gaa ggg caa ggc cgt ctg gat ggg gct ctc tgc tcc   1030
Leu Asp Phe Tyr Glu Gly Gln Gly Arg Leu Asp Gly Ala Leu Cys Ser
                215                 220                 225
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```
tac acg gag aag gac aag cag gcg tat ctg gag gca gcc tat gca gcc   1078
Tyr Thr Glu Lys Asp Lys Gln Ala Tyr Leu Glu Ala Ala Tyr Ala Ala
            230                 235                 240

ggc gtc cgc aat atc gag atg gag tcc tcg gtg ttt gcc gcc atg tgc   1126
Gly Val Arg Asn Ile Glu Met Glu Ser Ser Val Phe Ala Ala Met Cys
        245                 250                 255

agc gcc tgc ggc ctc caa gcg gcc gtg gtg tgt gtc acc ctc ctg aac   1174
Ser Ala Cys Gly Leu Gln Ala Ala Val Val Cys Val Thr Leu Leu Asn
    260                 265                 270

cgc ctg gaa ggg gac cag atc agc agc cct cgc aat gtg ctc agc gag   1222
Arg Leu Glu Gly Asp Gln Ile Ser Ser Pro Arg Asn Val Leu Ser Glu
275                 280                 285                 290

tac cag cag agg ccg cag cgg ctg gtg agc tac ttc atc aag aag aaa   1270
Tyr Gln Gln Arg Pro Gln Arg Leu Val Ser Tyr Phe Ile Lys Lys Lys
                295                 300                 305

ctg agc aag gcc tga gcgctgccct gcacctccgc agacctgctg tgatgacttg   1325
Leu Ser Lys Ala
            310

ccattaaaag cattgtccaa accc                                       1349
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<210> 2
<211> 310
<212> PRT
<213> Homo sapiens

<220>
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<222> (121)..(134)
<223> Xaa at position 121 = Glu or Lys; Xaa at position
134 = Val or Ile.

<400> 2

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  1               5                  10                  15

Cys Pro Val Arg Leu Leu Asn Pro Asn Ile Ala Lys Met Lys Glu Asp
             20                  25                  30

Ile Leu Tyr His Phe Asn Leu Thr Thr Ser Arg His Asn Phe Pro Ala
         35                  40                  45

Leu Phe Gly Asp Val Lys Phe Val Cys Val Gly Gly Ser Pro Ser Arg
     50                  55                  60

Met Lys Ala Phe Ile Arg Cys Val Gly Ala Glu Leu Gly Leu Asp Cys
 65                  70                  75                  80

Pro Gly Arg Asp Tyr Pro Asn Ile Cys Ala Gly Thr Asp Arg Tyr Ala
                 85                  90                  95
```

```
Met Tyr Lys Val Gly Pro Val Leu Ser Val Ser His Gly Met Gly Ile
            100                 105                 110

Pro Ser Ile Ser Ile Met Leu His Xaa Leu Ile Lys Leu Leu Tyr Tyr
        115                 120                 125

Ala Arg Cys Ser Asn Xaa Thr Ile Ile Arg Ile Gly Thr Ser Gly Gly
    130                 135                 140

Ile Gly Leu Glu Pro Gly Thr Val Val Ile Thr Glu Gln Ala Val Asp
145                 150                 155                 160

Thr Cys Phe Lys Ala Glu Phe Glu Gln Ile Val Leu Gly Lys Arg Val
                165                 170                 175

Ile Arg Lys Thr Asp Leu Asn Lys Lys Leu Val Gln Glu Leu Leu Leu
            180                 185                 190

Cys Ser Ala Glu Leu Ser Glu Phe Thr Thr Val Val Gly Asn Thr Met
        195                 200                 205

Cys Thr Leu Asp Phe Tyr Glu Gly Gln Gly Arg Leu Asp Gly Ala Leu
    210                 215                 220

Cys Ser Tyr Thr Glu Lys Asp Lys Gln Ala Tyr Leu Glu Ala Ala Tyr
225                 230                 235                 240

Ala Ala Gly Val Arg Asn Ile Glu Met Glu Ser Ser Val Phe Ala Ala
                245                 250                 255

Met Cys Ser Ala Cys Gly Leu Gln Ala Ala Val Val Cys Val Thr Leu
            260                 265                 270

Leu Asn Arg Leu Glu Gly Asp Gln Ile Ser Ser Pro Arg Asn Val Leu
        275                 280                 285

Ser Glu Tyr Gln Gln Arg Pro Gln Arg Leu Val Ser Tyr Phe Ile Lys
    290                 295                 300

Lys Lys Leu Ser Lys Ala
305                 310
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<220>
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<210> 4
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<210> 5
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<210> 6
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<400> 7
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<210> 8
<211> 38
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<223> Description of Artificial Sequence:forward primer

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